Annu. Rev. Cell Dev. Biol. 2000. 16:591–626

SUMO—NONCLASSICAL UBIQUITIN

Frauke Melchior
Max-Planck Institute for Biochemistry, Am Klopferspitz 18a, D-82152 Martinsried bei München, Germany; e-mail: melchior@biochem.mpg.de

Key Words Smt3, sentrin, Pic1, Ubl1, post-translational modification

■ **Abstract** SUMO (small ubiquitin-related modifier) is the best-characterized member of a growing family of ubiquitin-related proteins. It resembles ubiquitin in its structure, its ability to be ligated to other proteins, as well as in the mechanism of ligation. However, in contrast to ubiquitination—often the first step on a one-way road to protein degradation—SUMOlation does not seem to mark proteins for degradation. In fact, SUMO may even function as an antagonist of ubiquitin in the degradation of selected proteins. While most SUMO targets are still at large, available data provide compelling evidence for a role of SUMO in the regulation of protein-protein interactions and/or subcellular localization.

CONTENTS

INTRODUCTION

A multitude of mechanisms determine the in vivo function of proteins. Among them are the regulation of protein levels via control of expression levels and turnover and regulation of protein activity, and localization and/or interactions by constitutive or reversible post-translational modifications. These modifications, usually accomplished via enzymatic reactions, result, for example, in acetylation, methylation, phosphorylation, ADP ribosylation, carboxylation, adenylylation, and glycosylation or prenylation of amino acid side chains. Among these is also ubiquitination, a post-translational modification that was first discovered in 1987 and has since been extensively studied (Bonifacino & Weissman 1998, Hershko & Ciechanover 1998). Ubiquitination is the enzymatically catalyzed formation of an isopeptide bond between the C terminus of the 9-kDa polypeptide ubiquitin and ε-amino groups in lysines of the acceptor proteins. In principle, the modification is reversible, because the ubiquitin moiety can be removed from the acceptor molecule by deubiquitinating enzymes (isopeptidases). Ubiquitination is best known for its role in regulated protein degradation via the 26S proteasome. Commitment of a protein to the ubiquitin-dependent degradation pathway involves assembly of a polyubiquitin chain on the target, usually via isopeptide bonds between lysine 48 of one ubiquitin and the C-terminal glycine residue of the neighboring ubiquitin. Although tagging a protein with ubiquitin chains usually leads to its complete degradation, it is sometimes also used for controlled activation of the protein via limited proteolysis. In addition, monoubiquitination plays a role in receptor mediated endocytosis (Hicke 1997).

A number of proteins related to ubiquitin have been isolated over the years. These proteins fall into two groups, proteins that are not available for conjugation (e.g. Rad23, Dsk2p, Elongin B), and proteins that, like ubiquitin, are attached to other proteins (reviewed in Ciechanover 1998, Haas & Siepmann 1997, Hochstrasser 1998, Hodges et al 1998, Jentsch & Pyrowolaski 2000, Johnson & Hochstrasser 1997, Kretz-Remy & Tanguay 1999, Saitoh et al 1997b, Tanaka et al 1998, Vierstra & Callis 1999). To this second group belong the interferon-inducible ubiquitin

cross-reacting proteins UCRP/ISG15, Nedd8, and SUMO1 (small ubiquitin-related modifier), which are 36, 57, and 18%, respectively, identical to ubiquitin at their primary sequence. Whether the 21-kDa yeast protein Apg12 that is required for autophagy in yeast, also belongs to this family or whether it is the first member of a new group of attachable proteins is presently unclear. Although Apg12 does not show any discernible homology to ubiquitin at the level of its primary sequence, it is attached to Apg5 by a mechanism that closely resembles ubiquitination (Ohsumi 1999). It remains to be seen whether Apg12 is related to ubiquitin at a structural level.

The significance of ubiquitin-related proteins remained rather obscure until the first targets, the SUMO1 target RanGAP1 (Matunis et al 1996, Mahajan et al 1997), and the Nedd8/Rub1 target Cdc53 (Lammer et al 1998, Liakopoulos et al 1998) were discovered. Only than did it become clear that these proteins are not simply variations on the ubiquitin theme; they play important roles outside of protein degradation. Since then, ubiquitin-related proteins have been implicated in a vast number of cellular processes, including nuclear transport, signal transduction, apoptosis, autophagy, cell cycle control, and regulation of ubiquitin-dependent degradation. This review focuses on SUMO1 and its relatives; for information on Nedd8, Apg12, and other ubiquitin-related proteins the reader is referred to the reviews cited above.

SUMO—A PROTEIN WITH MANY NAMES

Within a few months of each other, several different groups independently identified cDNAs and/or genomic sequences for members of the mammalian SUMO family. Consequently, SUMO1 is now known under the names PIC1, Ubl1, sentrin, GMP1, Smt3c, and hSmt3, whereas its relatives SUMO2 and SUMO3 are also referred to as sentrin2 and sentrin3, or Smt3a and Smt3b. A brief review of their discovery is given below, and for the remainder of this review, the SUMO terminology is used.

The first reported member of the SUMO family was the *Saccharomyces cerevisiae SMT3*. It was cloned in a screen for suppressors of a temperature-sensitive allele of *MIF2*, a gene encoding a distant homolog of the mammalian centromere CENP-C protein (Meluh & Koshland 1995). Subsequently, several groups cloned different human and mouse homologs of *S. cerevisiae SMT3* by sequence homology and called them hSmt3 (Mannen et al 1996) or Smt3A, Smt3B, and Smt3C (Chen et al 1998, Lapenta et al 1997). At about the same time, SUMO1 was recognized as an interacting factor in a number of two-hybrid interaction screens. Shen and co-workers discovered SUMO1 (here called Ubl1 for ubiquitin-like protein 1) using either Rad51 or Rad52 as baits, but they could not demonstrate strong interactions between these proteins outside of the two-hybrid screen (Shen et al 1996b). Boddy and co-workers used pro myelocytic leukemia protein (PML), a RING finger protein involved in acute promyelocytic leukemia, as bait and isolated a small

ubiquitin-related protein, which they called PIC1 for PML interacting protein 1 (Boddy et al 1996). While they could not demonstrate a direct protein-protein interaction, they found that PML and PIC1 colocalize in the so-called POD or PD10 nuclear bodies. Using the death domain of the Fas/Apo receptor as bait in the yeast two-hybrid system, Okura and co-workers also identified SUMO1, which they called sentrin, after sentry, because it appeared to have a guardian function against cell death signaling (Okura et al 1996). Interestingly, this two-hybrid interaction requires a functional death domain. In vitro pull-down experiments indicated a direct interaction of SUMO1 with the functional, but not the defective, death, domains, but the physiological relevance of this interaction remains to be elucidated. Shortly thereafter two publications reported that the GTPase-activating protein RanGAP1 is covalently attached to a novel ubiquitin-related protein. Matunis & Blobel christened this protein GMP1, for GAP-modifying protein 1 (Matunis et al 1996), and we called it SUMO1, for small-ubiquitin-related modifier (Mahajan et al 1997).

SUMO GENES—FROM YEAST TO HUMANS

Members of the SUMO protein family appear to be present in protozoa, metazoa, plants, and fungi (yeasts). Figure 1 (see color insert) shows a phylogenetic tree of SUMO sequences of selected species. SUMO proteins from metazoa can be divided into two families: SUMO1 proteins and SUMO2/SUMO3 proteins. Within a given species, SUMO1 and SUMO2/3 proteins are about 50% identical to each other. Plant SUMO proteins and SUMO proteins from fungi and yeast fall into two groups distinct from both SUMO1 and SUMO2/3 proteins.

S. cerevisiae contains a single essential SUMO gene, *SMT3* (Johnson et al 1997, Meluh & Koshland 1995). In contrast, the *Schizosaccharomyces pombe* homolog *Pmt3* is not truly essential (Tanaka et al 1999). However, disruption leads to severe growth defects and phenotypes such as aberrant mitosis, increase in telomere length, and defects in chromosome segregation. Analysis of an expressed sequence tag (EST) and genomic databases indicates the presence of at least one SUMO family member in *Aspergillus nidulans*, *Botrytis cinerea*, *Dictyostelium discoideum*, *Candida albicans* and *C. trypanosoma*. The best-characterized plant SUMO protein is T-SUMO from tomato (Hanania et al 1999). It was identified through its interaction with ethylene-inducing xylanase from the fungus *Trichoderma viride* and has been implicated through antisense experiments in plant defense responses that lead to programmed cell death. ESTcDNAs encoding SUMO proteins have been found in libraries from many plant species (e.g. soja, maize, rice, pine tree, cedar, etc), and Vierstra & Callis report the presence of at least three different expressed SUMO species in Arabidopsis (Vierstra & Callis 1999, unpublished data). Zebrafish and *Xenopus laevis* have both SUMO1 and SUMO2/3 proteins, *Caenorhabditis elegans* has apparently only a SUMO1 family member (Choudhury & Li 1997), whereas silk worm expresses cDNAs for a SUMO2/3 protein. A *Drosophila melanogaster*

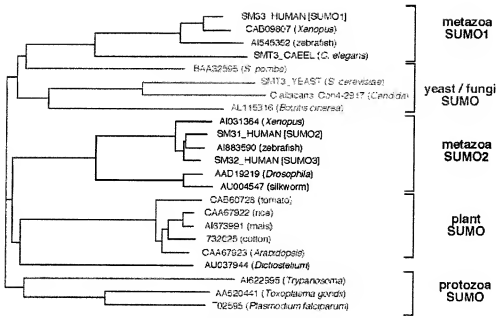

Figure 1 Phylogenetic tree of SUMO proteins. The calculation was done using the Vector NTI Suite™ software module AlignX™. Aligned cDNAs are listed by their accession numbers; additional information is provided in brackets.

SUMO2 protein has been described (Bhaskar et al 2000, Huang et al 1998), but there is currently no strong evidence for a SUMO1 in this organism. While Northern blot analysis revealed two different SUMO transcripts in Drosophila embryos, larvae, and adult tissue, it is currently unknown whether these derive from the same SUMO genes. Both mice and humans have at least three different SUMO proteins, SUMO1, SUMO2, and SUMO3. Although SUMO2 and SUMO3 are very similar at the amino acid level (87% sequence identity for the human proteins), they are only 47% identical to SUMO1. Transcripts for SUMO1, SUMO2, and SUMO3 can be detected in all human and mouse tissues, indicating that these proteins are ubiquitously expressed (Chen et al 1998, Howe et al 1998, Lapenta et al 1997, Mannen et al 1996, Shen et al 1996b). In addition to SUMO genes (Lapenta et al 1997, Shen et al 1996b) and a SUMO1-like gene (Collins et al 1998), three different processed pseudogenes for human SUMO1 and two pseudogenes for mouse SUMO1 have been identified (Howe et al 1998). Whether these pseudogenes are expressed remains to be seen.

Interestingly, SUMO3 was recently found in a bovine viral diarrhea virus (BVDV) (Qi et al 1998). It has been known for some time that ubiquitin insertions in some cytopathic BVDV play a key role in the conversion of a noncytopathic (nc) to a cytopathic (cp) viral strain. In the genome of these viruses, ubiquitin is integrated into the open reading frame of the nonstructural protein NS2-3. Cellular ubiquitin C-terminal hydrolases cleave the resulting fusion protein after the ubiquitin GlyGly motif, thereby releasing the C-terminal domain of NS2-3 (NS3). Expression of this truncated protein is the only known difference between the cp and nc forms of the virus. The integration of SUMO3 into the viral genome appears to play the same role as ubiquitin in the generation of NS3 and the resulting cytopathy of the virus.

SUMO1—STRUCTURE

Human SUMO1 shares only 18% sequence identity with ubiquitin. Nevertheless, NMR structure analysis (Bayer et al 1998) revealed that SUMO1 contains the characteristic $\beta\beta\alpha\beta\beta\alpha\beta$ ubiquitin-fold common to ubiquitin-like proteins (reviewed in Mayer et al 1998), and the three-dimensional folds of SUMO1 and ubiquitin can be superimposed (Figure 2). The GlyGly motif at the C terminus of both proteins, which is the site of attachment to enzymes and target proteins, is also positioned alike. In contrast, the surface charge distribution is significantly different between both proteins. It is therefore not surprising that the factors involved in SUMO conjugation are related to, but not identical to, ubiquitinating enzymes.

A key difference between ubiquitin and SUMO is the apparent inability of SUMO to be conjugated to itself. This is explained, at least in part, by the finding that neither lysine 48 nor other lysines used more rarely for ubiquitin chain formation (Pickart 1997) are conserved in SUMO. The most prominent difference between members of the SUMO family and other ubiquitin-related proteins (including ubiquitin) is a very flexible N-terminal extension present in SUMO.

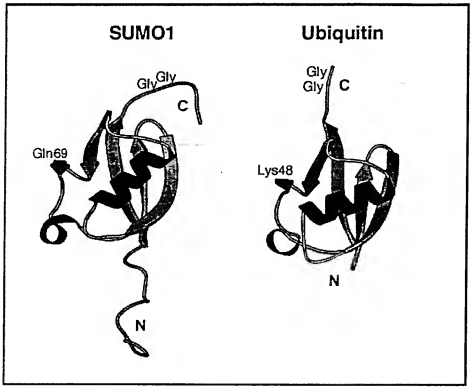

Figure 2 Comparison of the three-dimensional structures of SUMO1 and ubiquitin. Shown are the NMR structure of human SUMO1 (Bayer et al 1998) and the crystal structure of human ubiquitin (Vijay-Kumar et al 1987). The figure was modified from a file provided by J Becker.

This extension varies among different SUMO proteins from 11 to 35 amino acids and is reasonably well conserved within, but not between, different, SUMO families. The N-terminal extension of *S. cerevisiae* SUMO contains three repeats of the amino acid sequence motif (E/D)xKP, and one of these motifs is found in most SUMO proteins. In addition, N termini of all SUMO proteins are rich in charged amino acids, glycines, and prolines. Although the function of this extension is unknown, its characteristics make it an excellent candidate for specific protein/protein interactions.

ENZYMES INVOLVED IN SUMO LIGATION AND CLEAVAGE

Overview

Several of the enzymes involved in SUMO processing, conjugation, and deconjugation have been cloned and characterized in the last few years (Figure 3). As

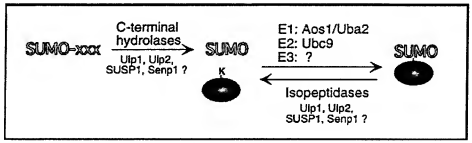

Figure 3 The SUMOlation pathway. After SUMO is proteolytically processed by C-terminal hydrolases, it serves as the substrate in the ATP-dependent formation of an isopeptide bond between the free carboxyl group of the C-terminal glycine in SUMO and the ε-amino group of a lysine in the acceptor protein. This reaction is mediated by the E1 enzyme Aos1/Uba2 and the E2 enzyme Ubc9. Whether E3 ligases are required for efficient modification in vivo is presently unknown. Cleavage of the isopeptide bond is mediated by isopeptidases.

can be expected from the high degree of structural similarity between SUMO and ubiquitin, the general mechanism of conjugation and the enzymes themselves are highly related. SUMO is first activated in an ATP-dependent reaction by formation of a thioester bond with an E1 (SUMO-activating) enzyme. In contrast to the ubiquitin-activating enzyme, the SUMO-E1 is a heterodimer consisting of a 40- and a 70-kDa subunit (Aos1 and Uba2, respectively). In a second reaction, SUMO is transferred to the SUMO conjugating (E2) enzyme Ubc9. In ubiquitination, E3 enzymes (ubiquitin ligases) ensure substrate specificity and are themselves also targets for significant regulation. While it is highly likely that similar factors also facilitate or mediate attachment of SUMO to its targets, such proteins are still unidentified. SUMO1 needs to be proteolytically processed prior to its conjugation and, like ubiquitination, SUMO modification is reversible. Four SUMO C-terminal hydrolases/isopeptidases have now been identified, Ulp1 and Ulp2 from *S. cerevisiae* and SUSP1 and Senp1 from humans. Interestingly, these enzymes are not related to their ubiquitin counterparts, instead they resemble viral cysteine-proteases in their catalytic core domain.

SUMO–Activating Enzyme Aos1/Uba2

S. cerevisiae SUMO-activating enzyme is a heterodimer consisting of the 38-kDa Aos1 and the 71-kDa Uba2 (Dohmen et al 1995, Johnson et al 1997). Both subunits are conserved from yeast to human and are essential in *S. cerevisiae*. *S. cerevisiae* Aos1 is 29% identical to the N-terminal region of the yeast ubiquitin E1 Uba1, whereas Uba2 shares 28% identity with 561 residues in the C-terminal half of Uba1. These regions of homology include two conserved nucleotide-binding motifs (one in Aos1, one in Uba2) and a consensus sequence around the active cysteine site in Uba2. In addition to this conserved domain, Uba2 contains an unrelated domain at its C-terminal end. This Uba2-specific domain does

not seem essential in *S. cerevisiae* because replacement of wild-type (wt) *UBA2* with a mutant lacking the last 115 amino acids was viable (del Olmo et al 1997).

S. cerevisiae Uba2 was identified serendipitously by Dohmen and co-workers in 1995, but its function as a SUMO-activating enzyme was only demonstrated two years later (Johnson et al 1997). Inspired by the homology of Uba2 to Uba1, the authors speculated that Uba2 was involved in SUMO conjugation. They exploited the intrinsic ability of E1 enzymes to form thioester bonds with their substrates in the presence of ATP. However, it was found that Uba2 alone was not sufficient for thioester bond formation; a second protein, present in the yeast extract, was also required. This protein, Aos1, assembles with Uba2 to form an active SUMO E1 enzyme in vitro. In vivo evidence for a role of Uba2 in SUMO conjugation came from investigation of a temperature-sensitive mutant of Uba2 that showed a striking decrease in SUMO conjugation at the restrictive temperature. At about the same time, Shayeghi et al described *rad31*, a *UBA1*-related gene from fission yeast that is required for DNA damage tolerance (Shayeghi et al 1997). Based on sequence homology and complementation studies, *rad31* is the fission yeast equivalent to *S. cerevisiae Aos1*. In contrast to *Aos1, S. pombe rad31* is not essential. However, disruption leads to slow growth and to a high-frequency loss of minichromosomes.

Two years later, three groups identified the human homologs of Aos1 (Aos1, Sua1, or SAE1) and Uba2 (Uba2 or SAE2). Desterro and co-workers (1999) followed a biochemical approach for the identification of a SUMO1 E1 enzyme; two other groups cloned Aos1 and Uba2 from ESTclones with sequence homology to their yeast counterparts (Gong et al 1999, Okuma et al 1999). Human Aos1 (38 kDa) and Uba2 (72 kDa) are 33 and 34% identical to their respective yeast homologs, form thioester bonds with SUMO1, SUMO2, and SUMO3 (Gong et al 1999), and are required for SUMO1 modification of IκBα (Desterro et al 1999), p53 (Rodriguez et al 1999) and RanGAP1 (Okuma et al 1999). There is some discrepancy regarding the apparent size of Uba2 in SDS PAGE. Uba2 was reported to migrate either at 70 or at 90 kDa in SDS PAGE, independent of the expression or translation systems used (del Olmo et al 1997, Desterro et al 1999, Dohmen et al 1995, Gong et al 1999, Okuma et al 1999). Although the reason for this discrepancy is not clear, one possibility could be that Uba2 is prone to limited proteolysis. Not much is known about expression pattern of the SUMO E1 enzyme, but basic local alignment search tool (BLAST) searches reveal EST cDNAs for both subunits from many different mammalian adult and embryo tissues, as well as from cultured cells, indicating that these proteins are ubiquitously expressed. Interestingly, Uba2 is a target for ubiquitination in yeast (Dohmen et al 1995); whether this is used to regulate efficiency of SUMOlation remains to be seen. The subcellular localization of Aos1 and Uba2 has not been studied in detail, but tagged Uba2 expressed from a high-copy plasmid was found enriched in the nucleus of *S. cerevisiae* (Dohmen et al 1995).

A specific binding partner for yeast Uba2 has been identified by two-hybrid interaction screening and co-immunoprecipitation. This binding partner is the poly(A) polymerase Pap1, which is responsible for the addition of the adenylate

tail to the 3′ end of mRNA (del Olmo et al 1997). A physiological significance of this interaction was suggested by in vitro polyadenylation experiments with Uba2-depleted extracts. In these extracts, polyadenylation activity is significantly reduced. While this may indicate that components of the polyadenylation machinery depend on SUMO modification, Pap1 itself does not appear to be posttranslationally modified.

SUMO–Conjugating Enzyme Ubc9

With 56% amino acid identity between the *S. cerevisiae* and the human protein (Seufert et al 1995, Watanabe et al 1996), Ubc9 is highly conserved from yeast to human, and mammalian Ubc9 can complement a temperature-sensitive (ts) mutation in the essential *S. cerevisiae* Ubc9 gene (Hateboer et al 1996, Kovalenko et al 1996, Yasugi & Howley 1996). The similarities between SUMO and ubiquitin are reiterated by the striking similarity between Ubc9 and the large family of ubiquitin-conjugating enzymes (Giraud et al 1998, Haas & Siepmann 1997, Tong et al 1997). Yeast Ubc9 shares 33 and 36% amino acid identity with the ubiquitin-conjugating enzymes *S. cerevisiae* Ubc4 and Ubc7, and the α-carbon backbones of human Ubc9 and plant Ubc1 can be superimposed in most part. However, like SUMO1 and ubiquitin, Ubc9 and ubiquitin E2s have very different surface charge distributions. Ubc9 contains a much more positively charged noncatalytic face (isoelectric points of Ubc9 and ubiquitin E2s are 8.7 versus 6.7 and lower). A recent NMR study, mapping the interface between Ubc9 and SUMO1, indicates that Ubc9 and SUMO1 have charged surfaces that are highly complementary in their electrostatic potential and hydrophobicity (Liu et al 1999). These findings nicely explain the striking specificity of Ubc9 for SUMO. The SUMO1 residues implicated in Ubc9 binding are well conserved within all SUMO family members. This fact, the ability of human Ubc9 to replace yeast Ubc9, and the finding that SUMO1, SUMO2, and SUMO3 all form thioesters with Aos1/Uba2 in vitro and can be attached to the same proteins in vivo (Gong et al 1999, Kamitani et al 1998b), strongly suggest that Ubc9 is involved in the conjugation of all SUMO family members.

S. cerevisiae Ubc9 was originally identified by Seufert and co-workers in 1995 based on its sequence similarity to known ubiquitin-conjugating enzymes. This similarity, together with the finding that a ts mutation in Ubc9 resulted in a G2/M arrest with high levels of the S-phase cyclin Clb5 and the M-phase cyclin Clb2, led to the assumption that Ubc9 is a bona fide ubiquitin-conjugating enzyme involved in cyclin degradation. Gene disruption of Ubc9 is lethal; although the spores germinate, they die after a few cell divisions. At the same time, al-Khodairy and co-workers identified *S. pombe* Ubc9 (hus5) in a screen for DNA damage checkpoint control (al-Khodairy et al 1995). Although hus5 did not seem to be directly involved in the checkpoint control, it was required for efficient recovery from DNA damage or S-phase arrest. Gene disruption of hus5 is not immediately lethal, but the disrupted mutants are severely impaired in growth and exhibit high levels of abortive mitosis. It was not until 1997 that Ubc9 was identified as a SUMO E2

enzyme when Johnson & Blobel (1997) identified yeast Ubc9 by biochemical means as a SUMO-conjugating enzyme and demonstrated its requirement for conjugation of SUMO to unknown targets in a yeast extract. Shortly thereafter, two groups demonstrated that Ubc9 is required for conjugation of SUMO1 to RanGAP1, at that time the only known SUMO1 target, in mammalian or *Xenopus* extracts (Lee et al 1998, Saitoh et al 1998). *Drosophila* and mammalian homologs of Ubc9 were identified by many laboratories, mainly through two-hybrid interaction screens in which Ubc9 was pulled out as a strong binding partner (see below) and through characterization of the *Drosophila* mutant *semushi* (Epps & Tanda 1998).

Ubc9 has been found in several different subcellular compartments. In yeast, overexpressed Ubc9-β galactosidase fusion protein was localized inside the nucleus (Seufert et al 1995), similar to endogenous *Drosophila* Ubc9 (Joanisse et al 1998). By indirect immunofluorescence we detected endogenous mammalian Ubc9 in the cytoplasm and nucleoplasm, but also enriched around the nuclear envelope. These results were confirmed by cell fractionation of rat liver, where the majority of Ubc9 fractionated with the cytosolic fraction (owing to its small size, monomeric Ubc9 is expected to leak out of the nucleus upon cell homogenization). However, a small fraction of Ubc9 is enriched in nuclear envelope fractions. This nuclear envelope–associated pool of Ubc9 is associated with RanGAP1 and/or RanBP2 (Lee et al 1998). Association of Ubc9 with RanGAP1 and RanBP2 has also been found in *X. laevis* oocyte extracts (Saitoh et al 1997a). Additionally, Ubc9 was detected at the synaptonemal complex in mouse prophase spermatocytes (Kovalenko et al 1996).

Human cells have a single Ubc9 gene that has been mapped by fluorescence in situ hybridization (FISH) to chromosome band 16p13.3. A predominant transcript of 1.3 kb and two minor transcripts at 3.3 (2.8 kb) and 6.4 kb (4.4 kb) were detected in many human tissues and cell lines, thus indicating that Ubc9 is ubiquitously expressed (Kovalenko et al 1996, Wang et al 1996, Watanabe et al 1996). In mouse, a single gene as well as three intronless processed pseudogenes, which may have arisen from retroposition of RNA molecules, were identified (Tsytsykova et al 1998). Two of these pseudogenes (ubc9-ψ and ubc9-ψ2) contain open reading frames with a single (W41G) or three (R8H, H20Y, and P28S) amino acid substitutions each. These amino acid exchanges all lie in the N-terminal helix of Ubc9, which appears to play some role in the recognition of SUMO or the SUMO E1 enzyme. Interestingly, these derivatives fail to rescue the Ubc9 ts phenotype in *S. cerevisiae* and do not interact with the Fas/Apo receptor in yeast two-hybrid interaction studies. Whether these proteins play a physiological role in mice remains to be shown, but their existence opens up several intriguing possibilities. On the one hand, they may interact preferentially with SUMO2 and SUMO3, whose conjugation patterns are significantly different from that of SUMO1 (see below); on the other hand, they could be involved in the modification of specific SUMO1 targets by selectively interacting with (hypothetical) E3 SUMO ligases or other accessory factors.

SUMO–Ligating Enzymes?

Ubiquitin-ligating (E3) enzymes are defined as enzymes that bind, directly or indirectly, specific protein substrates and promote the transfer of ubiquitin, directly or indirectly, from a thiolester intermediate to amide linkages with proteins or polyubiquitin chains (Hershko & Ciechanover 1998). This rather broad definition became necessary after the realization that E3 enzymes fall into distinct subclasses that promote ubiquitination via a variety of different mechanisms, with only some involving thioester bond formation between ubiquitin and the E3 enzyme. Although SUMO-ligating enzymes have not yet been identified, several observations strongly suggest their existence. First, in vitro modification of the SUMO1 targets RanGAP1, IκBα, and p53 with purified or recombinant E1 and E2 enzymes (Desterro et al 1998, Okuma et al 1999, Rodriguez et al 1999; U Gärtner & F Melchior, unpublished data) is very inefficient compared with RanGAP1 SUMOlation in complete cell extracts (Mahajan et al 1997). Second, if Ubc9 itself recognized its targets efficiently, one would expect at least some sequence homology between different SUMO targets. For three targets relatively short domains that are sufficient for SUMO1 modification have been defined: amino acids 400–595 in mouse RanGAP1 (Matunis et al 1998), amino acids 294–393 in p53 (Gostissa et al 1999), and amino acids 1–256 in IκBα (Desterro et al 1998). No obvious sequence homology has been depicted among these protein fragments. Third, although the human SUMO1 target topoisomerase I can be efficiently modified with Smt3 when expressed in *S. cerevisiae* (Mao et al 2000; see below), this is not the case for mouse RanGAP1 (A Gast & F Melchior, unpublished data), which suggests that yeast lacks a RanGAP1-specific factor for modification. Perhaps the best argument for the existence of substrate-specific ligating enzymes comes from the observation that SUMO1 and SUMO2/3 are conjugated to different targets in vivo and that their conjugation appears to be differentially regulated (Saitoh & Hinchey 2000; see below).

SUMO C-Terminal Hydrolases and Isopeptidases

Ubiquitin C-terminal hydrolases and/or isopeptidases (deubiquitinating enzymes, DUBs) are thiol proteases that hydrolyze ester, thiol ester, and amide bonds to the carboxyl group of Gly76 of ubiquitin. Their physiological functions involve processing of ubiquitin precursors, salvage of ubiquitin that has been trapped by reaction with small cellular nucleophiles such as thiols and amines, disassembly of polyubiquitin chains and recycling of ubiquitin from late proteolytic intermediates, as well as regulation of the ubiquitination state of proteins (Chung & Baek 1999, Wilkinson 1997). Like ubiquitin, SUMO proteins are expressed as precursors that need to be proteolytically processed by C-terminal hydrolases to make the C-terminal Gly Gly motif available for conjugation. While there is currently no evidence for the existence of poly-SUMO chains that require cleaving by isopeptidases, SUMO-protein conjugates are highly susceptible to deconjugation

in cell extracts and different subcellular fractions, indicating the presence of several isopeptidases.

At the present time, two related SUMO processing enzymes have been found in *S. cerevisiae*, 72-kDa Ulp1 (Li & Hochstrasser 1999) and the 117-kDa Ulp2/Smt4p (Li & Hochstrasser 2000; I Schwienhorst, E Johnson, RJ Dohmen, personal communication). Two human enzymes have recently been cloned owing to their weak homology to Ulp1, and analysis of EST cDNAs indicates the presence of a large family of related proteins. The characterized proteins are the 73-kDa protein Senp1 (Gong et al 2000) and the 126-kDa protein SUSP1 (Kim et al 2000). In addition, a 30-kDa SUMO1 C-terminal hydrolase has been biochemically enriched from bovine brain (Suzuki et al 1999). All five proteins appear to have C-terminal hydrolase activity, and Ulp1, Ulp2, Senp1, and the 30-kDa protein also cleave isopeptide bonds in vitro. Like the deubiquitinating enzymes, Ulp1, Ulp2, Senp1, and SUSP1 are cysteine proteases, but they are unrelated in sequence to DUBs. Instead, as Li & Hochstrasser discovered, these proteins share some homology with the adenovirus L3 protease, a cysteine protease that cleaves viral protein precursors after a GlyGlyX motif. The sequence similarity between Ulp1, SUSP1, and Senp1 is restricted to a 200-amino acid area surrounding the 90-amino acid core domain common to viral proteases and Ulp-related sequences from yeast to human. Four key catalytic residues of the adenovirus protease are conserved in all Ulp1-related sequences, and mutation of two of these residues (Cys 580 Ser and His 514 Arg) in *S. cerevisiae* Ulp1 leads to loss of its C-terminal hydrolase activity (Li & Hochstrasser 1999). These findings suggest that Ulp proteins may use a catalytic mechanism similar to that of the viral proteases. Several viral infections seem to interfere with SUMOlation of proteins such as PML (see below), and one might speculate that viral proteases play a direct role in this. It will be extremely interesting to test their activity toward SUMOlated proteins.

Li & Hochstrasser (1999) identified *S. cerevisiae* Ulp1 by screening a genomic expression library for activity against a His6-ubiquitin-SUMO-HA fusion protein. Upon sequencing of Ulp1, they realized that it contains weak but significant homology to Smt4. Like SUMO1, this protein had been found in a high-copy-suppressor screen for Mif2, but its function remained obscure (Meluh & Koshland 1995). Biochemical analysis confirmed that Smt4 is in fact a SUMO hydrolase and it was thus renamed Ulp2 (Li & Hochstrasser 2000). At the same time, Ulp2 was identified in a screen for spontaneous suppressors of a *uba2*-ts strain (I Schwienhorst, E Johnson, RJ Dohmen, personal communication). Analysis of a temperature-sensitive mutant and gene disruption studies indicates that Ulp1 is an essential protein required for deconjugation of SUMO targets. *Ulp1*-ts cells arrest at the G2/M boundary when shifted to the restrictive temperature, suggesting that at least one of its substrates needs to be deconjugated for progression through mitosis. In addition, Ulp1 seems to be involved in processing full-length SUMO because the lethality of Ulp1 disruption can be partially overcome by overexpressing mature SUMO. In contrast to Ulp1, Ulp2 is not absolutely essential. However, disruption leads to pleiotropic phenotypes including slow and temperature-sensitive growth,

defects in cell morphology, chromosome instability, and sporulation defects. Like Ulp1, Ulp2 appears to function as an isopeptidase in vivo, but it seems to play only a minor role in processing SUMO. Interestingly, disruption of Ulp2 in a *uba2*-ts background allows normal growth, indicating that SUMO conjugation and deconjugation must be balanced for cells to grow normally (I Schwienhorst, E Johnson, RJ Dohmen, personal communication). Somewhat puzzling is the finding that *ulp1*-ts defects can be partially overcome by deletion of *ULP2*. One possible explanation is that Ulp1 and Ulp2 control the modification levels of proteins in opposing pathways (Li & Hochstrasser 2000).

Interestingly, Ulp1 and Ulp2 in yeast, and SUSP1 and Senp1 in mammalian cells, localize to different subcellular compartments. Both Ulp2 and Senp1 are predominantly intranuclear; Ulp1 appears to be concentrated around the nuclear envelope, and SUSP1 is found in the cytoplasm (Gong et al 2000, Kim et al 2000, Li & Hochstrasser 2000). Overexpression experiments suggest that Senp1 cleaves SUMOlated PML and most unknown SUMO conjugates but not SUMOlated RanGAP1, consistent with its localization in the nucleus (Gong et al 2000).

DYNAMIC MODIFICATION BY SUMO

SUMO Conjugation in Yeast

In *S. cerevisiae*, SUMO conjugation seems to be essential for viability as *SMT3*, *UBA2*, *AOS1*, *UBC9*, and *ULP1* are all essential genes. Moreover, mutations of the catalytic cysteines in both Ubc9 and Uba2, as well as deletion of the C-terminal Gly Gly motif in SUMO, are not viable (Dohmen et al 1995, Johnson et al 1997, Li & Hochstrasser 1999, Seufert et al 1995). Temperature-sensitive alleles of *SMT3*, *AOS1*, *UBA2*, and *UBC9* lead to an accumulation of G2/M cells at the restrictive temperature, indicating that conjugation of SUMO to at least one protein may be important for progression through mitosis. A similar G2/M arrest was observed for yeast carrying a ts-allele of Ulp1 (Li & Hochstrasser 1999). This arrest was not overcome by simultaneous overexpression of processed SUMO, suggesting that cleavage of a SUMO-protein conjugate rather than lack of free SUMO for conjugation was the reason for the cell cycle arrest in this strain. Together, these findings indicate that both conjugation and deconjugation of SUMO are required for progression through mitosis.

Consistent with a dynamic role of the SUMO modification is the observation that SUMO conjugation patterns differ significantly in cultures growing exponentially or approaching stationary phase and in cultures arrested in different cell cycle phases (Li & Hochstrasser 1999, Takahashi et al 1999). By immunofluorescence analysis SUMO localizes to the nucleus in all stages of the cell cycle, but most strikingly is localized to a ring at the bud neck only during mitosis. This ring persists through anaphase and disappears abruptly at cytokinesis. SUMO staining at the bud neck is (predominantly) from SUMOlated septins Cdc3, Cdc11, and

Shs1/Sep7 (Johnson & Blobel 1999, Takahashi et al 1999; see below). However, septin modification does not seem to be required for the G2/M transition, indicating that the essential SUMO target(s) is still at large.

Another mitotic function in which SUMO has been implicated (albeit much more indirectly) is chromosome segregation. SUMO (*SMT3*) as well as Ulp2 (*SMT4*) were both originally identified as high-copy suppressors of a mutant in the centromere protein Mif2 (Meluh & Koshland 1995), and yeast Ubc9 interacts in two-hybrid screens with Cbf3p, a subunit of the centromere DNA-binding core complex CBF3 (Jiang & Koltin 1996). A putative role for SUMO in chromosome segregation is further supported by studies in fission yeast. In contrast to *S. cerevisiae, Schizosaccharomyces pombe* does not absolutely require SUMOlation for viability. Neither *pmt3* (the gene for SUMO) nor *rad31* (*S. pombe* Aos1), nor *hus5* (the gene for *S. pombe* Ubc9) are truly essential (al-Khodairy et al 1995, Shayeghi et al 1997, Tanaka et al 1999). However, disruption of any of these three genes leads to high levels of abortive mitosis and loss of minichromosomes, defects that would be consistent with a role of these genes in chromosome segregation. Moreover, GFP-SUMO, which can functionally replace the wt protein, seems to partially colocalize with spindle pole bodies in interphase but not in prometaphase and metaphase (Tanaka et al 1999). In addition to defects in chromosome segregation, disruption of *S. pombe* SUMO also leads to a striking increase in telomere length that can be reversed by reintroduction of SUMO. However, because of the large number of unknown proteins that appear to be conjugated to SUMO both in *S. cerevisiae* and in *S. pombe*, any of the phenotypes described above could be rather indirect. Without identification of specific targets, we are far from understanding the in vivo function of this post-translational modification.

SUMO1 and SUMO2/3 Conjugation in Mammalian Cells

At least three SUMO proteins are expressed in mammalian cells: SUMO1 and the highly related proteins SUMO2 and SUMO3. Whereas the in vitro data suggest that all three are conjugated via the Aos1/Uba2 and Ubc9 pathways, their conjugation patterns are clearly distinct (Saitoh & Hinchey 2000 and references therein). A large number of predominantly high-molecular-weight proteins are conjugated to SUMO1 in mammalian cells, and very little free SUMO1 is detectable. The majority of the SUMO conjugates reside inside the nucleus, in part concentrated in different nuclear speckles, and at the nuclear envelope. In addition, SUMO1 can be detected at the nuclear envelope and to a smaller extent in the cytoplasm. Whether the cytoplasmic signals derive exclusively from free SUMO1 or also represent some cytoplasmic SUMO conjugates remains to be seen. In contrast to the findings in yeast, the overall pattern of SUMO1 conjugation in mammalian cells does not appear to change dramatically during and upon exit of the cell cycle. Moreover, different stress treatments such as heat shock, oxidative, or osmotic stress do not alter the overall SUMO1 conjugation pattern. However, individual proteins may well be affected by the cell cycle state or by stress. In fact, for the SUMO target PML, cell cycle-dependent SUMOlation has been described (Everett

et al 1999). Moreover, the level of SUMO1 modification of topoisomerase I and of mdm2 changes dramatically upon DNA damage (Mao et al 2000, Buschmann et al 2000; see below).

Quite a different picture emerges for SUMO2/SUMO3 conjugation. With the help of antibodies that specifically recognize SUMO1 or SUMO2/3, Saitoh & Hinchey (2000) found that a large amount of free SUMO2/3 but very few conjugates are present in cells kept at normal growth conditions. This was dramatically changed upon heat shock and oxidative or ethanol stress. Massive conjugation to predominantly high-molecular-weight proteins was visible 5 min after elevation of the temperature to 42°C, indicating that post-translational mechanisms are responsible for the activation of this pathway. These findings further the idea that SUMO1 and SUMO2/3 are not interchangeable proteins but play distinct roles in the modification of specific targets. This is also supported by the finding that the bona fide SUMO1 target RanGAP1 protein does not appear to be conjugated to SUMO2/3 at significant levels in vivo (Saitoh & Hinchey 2000). This last result is in apparent conflict with a report that showed conjugates of RanGAP1, as well as PML, with all three members of the SUMO family in transfection experiments (Kamitani et al 1998b). However, this may have been a consequence of overexpression of SUMO proteins.

SUMO TARGETS

A major difficulty in identifying SUMO targets has been that these proteins are rapidly deconjugated by isopeptidases upon cell lysis in nondenaturing buffers. An exception is RanGAP1, which appears to be conjugated even more efficiently in vitro than in vivo. Moreover, due to the bulky modification, antibodies may fail to recognize the modified proteins. Finally, only a tiny subpopulation of most targets seem to be conjugated to SUMO at steady-state levels in vivo. This may explain why even for the extensively studied proteins IκBα and p53, this post-translational modification has long been overlooked. Experimental conditions that have eventually allowed identification of the modification are lysis of cells under denaturing conditions and use of *N*-ethylmaleimide or iodoacetimide to inhibit isopeptidases. Overexpression of SUMO1 or Ubc9 in cells increases the level of SUMOlation and has therefore also helped to detect the modification of specific targets. Of the large number of SUMOlated proteins detected by Western blot analysis, 19 have been identified (Table 1). A brief description of these proteins follows below; for a more detailed review on the modification of PML and IκBα see Kretz-Remy & Tanguay 1999.

RanGAP1

The first identified target for SUMO1 modification was RanGAP1, the GTPase-activating protein for the Ras-related GTPase Ran that is involved in nucleocytoplasmic transport (Mahajan et al 1997, Matunis et al 1996). RanGAP1 is present

TABLE 1 Bona fide SUMO targets

Target	Two-hybrid with	PEST sequence[a]	References
RanGAP1		+	Mahajan et al 1997, Matunis et al 1996; Saitoh et al 1998
RanBP2		+	Saitoh et al 1998
PML	Ubc9 SUMO1	+	Boddy et al 1996, Duprez et al 1999, Kamitani et al 1998b, Müller et al 1998, Sternsdorf et al 1997
Sp100		+	Sternsdorf et al 1997; Sternsdorf et al 1999
IκBα	Ubc9	+	Desterro et al 1998
D. melanogaster Dorsal	Ubc9	+	Bhaskar et al 2000
p53	Ubc9	+	Gostissa et al 1999, Müller et al 2000, Rodriguez et al 1999, Shen et al 1996a
c-Jun	Ubc9	+	Göttlicher et al 1996, Müller et al 2000
HIPK2	Ubc9	+	Kim et al 1999
S. cerevisiae Cdc3		−	Johnson & Blobel 1999, Takahashi et al 1999
S. cerevisiae Cdc11		−	Johnson & Blobel 1999
S. cerevisiae Sep7/Shs1		0	Johnson & Blobel 1999
hCMV IE1		+	Müller & Dejean 1999
hCMV IE2	Ubc9 SUMO1 SUMO3	+	Hofmann et al 2000
D. melanogaster Tramtrack		+	Lehembre et al 2000
Topoisomerase I		−	Mao et al 2000
Glut1 (?)	UBC9	−	Giorgino et al 2000
Glut4 (?)	UBC9	0	Giorgino et al 2000
Mdm2		+	Buschmann et al 2000

[a]As determined by the PEST-find algorithm developed by Rechsteiner & Rogers (www.at.embnet.org/embnet/tools/bio/PESTfind/).

+ indicates at least one PEST-like sequence with a score above +5.

0 indicates at least one PEST-like sequence with a score between 0 and 5.

− indicates a score below 0.

(?) see text.

in the cytoplasm and at the cytoplasmic filaments of the nuclear pore complexes, where it forms a stable complex with the 358-kDa protein RanBP2/Nup358. It is this pore complex-associated RanGAP1 and not the cytoplasmic pool that functions in nucleocytoplasmic transport (Mahajan et al 1997). The association of RanGAP1 with RanBP2 depends on the modification of RanGAP1 with a single SUMO1 molecule that is attached to lysine 526 in RanGAP1. While a 100-amino acid domain of RanGAP1 is sufficient for SUMO modification (amino acids 502–592), an additional 83 amino acids of RanGAP1 are required for subsequent association of SUMOlated RanGAP1 with RanBP2 (Matunis et al 1998). This indicates that necessary binding information for RanBP2 resides in RanGAP1. SUMO1 itself does not bind to RanBP2. Whether it contributes to the RanBP2 binding or alters the conformation of RanGAP1 such that a hidden binding site becomes accessible remains to be seen. Activities that reverse RanGAP1 SUMOlation have been found in both cytosolic and nuclear envelope extracts (Mahajan et al 1997, Matunis et al 1996), and in cytosolic extracts RanGAP1 undergoes rapid cycles of modification and demodification (T Büsgen & F Melchior, unpublished data). In contrast, RanGAP1-SUMO1 that is associated with RanBP2 at nuclear pore complexes is not subject to rapid demodification in vitro (Mahajan et al 1998), indicating that association with RanBP2 protects SUMOlated RanGAP1 from isopeptidases. Mechanisms that would either allow access of isopeptidases to NPC-associated RanGAP1 or lead to a disruption of the RanBP2/RanGAP-SUMO1 complex are not known. Interestingly, yeast RanGAP is exclusively cytoplasmic and not SUMOlated because it lacks the C-terminal 20-kDa domain of its mammalian counterpart. This suggests that the SUMO1-dependent localization of RanGAP1 to the pore complexes in higher eukaryotes is not essential for the basic mechanism of nucleocytoplasmic transport. Rather, it may increase efficiency of the translocation machinery by placing RanGAP1 at the pore complex. An intriguing possibility we are currently pursuing is that SUMO1-dependent localization of RanGAP1 at RanBP2 may be a controlled process involved in the regulation of nuclear protein import rates.

RanBP2

The binding partner for SUMOlated RanGAP1, the 358-kDa nuclear pore complex protein RanBP2, also seems to be a target for SUMOlation. This has been reported by Saitoh and co-workers for RanBP2 in *X. laevis* egg extracts. They initially found that Ubc9 coprecipitates with RanBP2 and RanGAP1 and subsequently showed that Ubc9 is required for conjugation of RanGAP1 with SUMO1 (Saitoh et al 1998). During the course of these studies, they observed that RanBP2 immunoprecipitated from *X. laevis* extracts reacts with anti-SUMO1 antibodies. Subsequent identification of a RanBP2 subdomain that is SUMOlated in vitro indicated that this domain overlaps with the Ubc9-binding site (Saitoh et al 1998), as well as with the site that binds to SUMOlated RanGAP1 (Matunis et al 1998). The function for the RanBP2 modification is unknown, and there is as yet no evidence for an in vivo modification of RanBP2. SUMOlation of RanBP2 is not required

for its interaction with SUMOlated RanGAP1 or with Ubc9 because RanBP2 in isolated rat liver nuclear envelopes is unmodified but nevertheless coprecipitates with both proteins (Lee et al 1998).

PML

PML is a interferon-inducible RING finger-containing nuclear phosphoprotein of unknown function that forms homodimers via its coiled-coil domain. Its name stems from the finding that the PML gène has undergone a fusion with the retinoic acid receptor α gene in the majority of patients with acute promyelocytic leukemia (APL) (reviewed in Lin et al 1999). PML is highly enriched in subnuclear structures called PODs, PML nuclear bodies or PD10 bodies, but is also present in the nucleoplasm and in the cytoplasm (Sternsdorf et al 1997 and references therein). The chimeric PML-RARα protein, which is expressed in cell lines derived from patients with acute promyelocytic leukemia, alters the distribution of PML from nuclear bodies into microdispersed particles. Normal distribution of PML in these cells can be reconstituted upon application of retinoic acid or As_2O_3, probably because of selective degradation of the retinoic acid receptor-PML chimera. While the exact function of PODs remains an enigma, increasing evidence links them to the replication and transcription of DNA viruses and interferon-induced cellular defense mechanisms (reviewed in Maul 1998). PML was first implicated in the SUMOlation pathway through its interaction with SUMO1 in two-hybrid interaction screens and the finding that both proteins colocalized in PODs (Boddy et al 1996). Subsequently, two groups (Müller et al 1998, Sternsdorf et al 1997) provided direct evidence for the presence of SUMO1-PML conjugates. Mutagenesis of PML indicates the existence of at least three lysine residues that can serve as the SUMO1 acceptor sites (Duprez et al 1999, Kamitani et al 1998a). The analysis of PML SUMOlation has been complicated by very low levels of PML expression, the existence of several PML isoforms, and the presence of multiple distinct SUMO-PML conjugates. Cell fractionation experiments indicate that only POD-associated PML is modified with SUMO1, but the soluble pool of PML may have been deconjugated by isopeptidases in the course of the experiment. SUMOlated forms of a cytosolic PML mutant can be detected by Western blot analysis upon overexpression of SUMO1 (Kamitani et al 1998a), indicating that modification of PML can take place in the cytoplasm. Work by Müller & Dejan (1999) supports the notion that SUMO1 plays a role in the localization of PML to PODs. For example, they correlated loss of SUMOlation upon transfection with the viral proteins ICP0 or CMV IE1 (see below) to a relocalization of PML from PODs to the nucleoplasm. On the other hand, two groups report that a PML mutant lacking all SUMOlation sites still accumulates in POD-like structures (Ishov et al 1999, Li et al 2000). This may suggest that PML is only SUMOlated upon binding to the PODs. However, these experiments were carried out in the background of endogeneous PML, and POD-targeting may have been

accomplished by heterodimerization of the mutant with wt PML. Localizing this mutant in PML-/- cells should finally settle this question. A potential binding partner for SUMOlated PML is the DNA-binding protein Daxx, which is recruited to PODs by wt PML, but not the SUMOlation-deficient mutant (Ishov et al 1999, Li et al 2000).

Treatment of cells with calyculin A, a strong inhibitor of serine/threonine phosphatases 1 and 2A, inhibits PML SUMOlation (Müller et al 1998). It is, however, unclear whether this inhibition is caused by hyperphosphorylation of PML or of a protein involved in PML modification or demodification. Treatment of cells with As_2O_3 leads to the appearance of an anti-SUMO1 reactive high-molecular-weight smear, which has been interpreted as poly-SUMOlated PML (Müller et al 1998). However, As_2O_3 treatment and viral infection have previously been shown to lead to PML degradation, presumably by the ubiquitin/proteasome pathway (Chelbi-Alix & de The 1999 and references therein; Everett et al 1998), and SUMO1 lacks lysine 48 that is normally used for ubiquitin chains. Therefore, we consider it more likely that the observed high-molecular-weight bands are derived from polyubiquitination of PML-SUMO conjugates. It will be interesting to determine whether SUMOlation is a prerequisite of subsequent ubiquitination.

SP100

Like PML, Sp100 is an interferon-inducible protein highly enriched in PML bodies. Several alternatively spliced Sp100 proteins are expressed, but their physiological functions remain to be elucidated. Sternsdorf and co-workers demonstrated that the 54-kDa Sp100 can be modified by SUMO1 and mapped a single lysine residue, Lys297R, as the SUMO1 acceptor site. Interestingly, mutation of this lysine to arginine completely abolished SUMOlation of Sp100 but did not affect the localization of Sp100 in PODs (Sternsdorf et al 1999). At first glance, this indicates that SUMOlation of Sp100 is not required for nuclear localization and subsequent targeting to the PODs. However, Sternsdorf and co-workers also demonstrated that the POD targeting sequence of Sp100 includes a homodimerization domain. Targeting of the mutant protein could therefore also be mediated via dimerization with endogenous Sp100-SUMO1 conjugate. Inhibition of Sp100 nuclear import by a single point mutation in the nuclear localization site also abolished its SUMOlation. That this in fact was the result of aberrant localization was nicely demonstrated upon fusion of this mutant to an unrelated nuclear localization sequence. Whether this indicates, as the authors suggest, that nuclear localization is a prerequisite to the modification of Sp100 remains to be seen. An alternative interpretation of the results posits that the modification could well take place in the cytoplasm but nuclear import and/or subsequent association with PODs would be required for the protection of SUMOlated Sp100 from isopeptidases.

Cytomegalovirus IE1 and IE2

The cytomegalovirus immediate early proteins (IE1 and IE2) are nuclear phosphoproteins transcribed from the major immediate-early gene of human cytomegalovirus. These proteins are thought to play key roles in initiating and maintaining hCMV gene regulation pathways in both lytic and latent infections. The 72-kDa IE1 protein belongs to an increasing number of viral proteins (among them are also adenovirus E4 ORF3 and the HSV protein ICP0) that transiently localize with and subsequently disrupt PML bodies (reviewed in Maul 1998). In an attempt to dissect the molecular mechanisms underlying this disruption, Müller & Dejan (1999) discovered that CMV IE1 itself (but not HSV ICP0) is a target for SUMOlation. IE1-L174P is a mutant form of IE1 that neither interacts with PODs nor leads to their disruption. This mutant was also efficiently modified by SUMO1, indicating that SUMOlation of IE1 is not sufficient for its interaction with the PODs (Müller & Dejean 1999).

At about the same time, Hofmann and coworkers identified SUMO1, SUMO3, and Ubc9 as binding partners for the IE2-p86 protein in a yeast two-hybrid screen (Hofmann et al 2000). They further demonstrated that IE2-p86 can be modified by SUMO1 or SUMO3 at either one of two different lysine residues. Immunolocalization and reporter assays indicate that SUMOlation of IE2-p86 is critical for the transactivation capacity of IE2, but not for its localization to PODs.

IκBα

NFκB transcription factors are primarily regulated by association with IκB proteins. In most cells NFκB exists in the cytoplasm in an inactive complex bound to IκBα. Upon induction of the NFκB pathway by TNFα or phorbol esters, for example, IκBα is phosphorylated at serines 32 and 36, ubiquitinated at lysine 21, and subsequently degraded by the proteasome. This releases NFκB and allows its translocation into the nucleus. Among the genes that are induced by binding of NFκB is its inhibitor IκBα, which in an autoregulatory loop enters the nucleus upon synthesis, binds to, and subsequently exports NFκB. Recent findings by Desterro and co-workers add an interesting twist to the already complex regulation of NFκB activity (Desterro et al 1998). They report that IκBα can be SUMOlated at the same lysine that is also ubiquitinated. However, in contrast to ubiquitination of IκBα, SUMOlation is inhibited by phosphorylation. These findings, together with the observation that SUMOlated IκBα is resistant to TNFα-induced degradation, indicate that SUMO1 has the potential to function as an antagonist of ubiquitin. This is an intruiging possibility that could have major implications for many substrates of ubiquitination. However, whether this is its (only) role in the modification of IκBα is not yet clear. Overexpression of SUMO1 and/or Ubc9 apparently leads to SUMOlation of only a fraction of the total IκBα; nevertheless, it has a significant effect on NFκB-dependent transcription in reporter assays (Desterro et al 1998). This effect cannot simply be explained with an antagonist model.

Other functions of SUMO1 that would be more consistent with low steady-state levels of SUMOlated IκBα could, for example, involve regulation of IκBα localization, IκBα interactions, or protection of a specific (e.g. the nuclear) pool of IκBα. Interpretation of the NFκB-dependent reporter assay is additionally complicated by the possibility that components upstream of IκBα are also affected by overexpression of SUMO1 and Ubc9; for example, the TNFα receptor, which interacts with both Ubc9 (Saltzman et al 1998) and SUMO1 (Okura et al 1996). Although the idea that SUMO1 functions as a ubiquitin antagonist is very appealing, it is not yet clear whether this is what it is doing for IκBα.

Dorsal

Interestingly, not only IκBα but also the transcription factor NFκB itself may be a target for SUMOlation. This has been suggested by recent studies of the *Drosophila* transcription factor Dorsal (Bhaskar et al 2000), which initiates dorsoventral patterning in the embryo. Prior to activation of the Dorsal pathway, Dorsal is retained in the cytoplasm through its interaction with the IκBα–related protein Cactus. Upon phosphorylation, Cactus is degraded by the ubiquitin/proteasome machinery and Dorsal is translocated into the nucleus. Bhaskar and co-workers identified *Drosophila* Ubc9 as an interacting partner for Dorsal and demonstrated SUMOlation of Dorsal upon overexpression of either *Drosophila* SUMO2 or Ubc9 in S2 cells. Transient transfection of a Dorsal-GFP fusion protein leads to its accumulation in the nuclei of S2 cells. This intranuclear accumulation can be prevented by coexpression of Cactus. However, if Ubc9 is simultaneously overexpressed, the cytoplasmic retention of Dorsal by Cactus is abolished. Consistent with these findings are results of reporter assays with a Dorsal-responsive promoter. In the presence of Cactus, Dorsal-induced transcription is reduced to basal levels. Transfection of Ubc9, SUMO2, Aos1/Uba2, or combinations of these not only reverse the inhibition by Cactus but stimulate transcription beyond the levels reached without Cactus. In conclusion, these data suggest a role for SUMOlation in either nuclear translocation or retention of Dorsal.

p53

The tumor suppressor p53 is a transcription factor that can inhibit cell cycle progression and/or induce apoptosis (reviewed in Levine 1997). p53 is normally expressed at very low levels and exhibits a short half life, but it accumulates rapidly under a variety of stress conditions such as DNA damage, heat shock, or reactive oxygen species. The rapid turnover of p53 is accomplished by the ubiquitin-proteasome pathway, to which it is targeted through interaction with Mdm2. Phosphorylation of the p53 N terminus reduces its affinity for Mdm2 and thereby leads to its stabilization. Several types of post-translational modifications of the C terminus of p53 have been implicated in its activation, e.g. phosphorylation, acetylation, and glycosylation (Giaccia & Kastan 1998). Three groups recently reported that the C terminus of p53 can also be modified by SUMO1

(Gostissa et al 1999, Rodriguez et al 1999, Müller et al 2000). The C-terminal 100 amino acids of p53, which include the acceptor lysine 386, are sufficient for SUMOlation; whereas mutation of lysine 386 does not prevent p53 ubiquitination, it is not known whether SUMO modification influences p53 ubiquitination. In reporter assays, overexpression of SUMO1 and/or Ubc9 enhances p53-dependent transcription. Moreover, an endogenous target for p53-dependent transcription, the cyclin/Cdk kinase inhibitor p21, was apparently upregulated upon cotransfection of SUMO1 and Ubc9 in Saos-2 cells (Gostissa et al 1999). Taken together, these reports provide strong evidence for the ability of SUMO1 to modify p53 and suggest that this modification plays a role in the activation of p53. However, considering the large number of potential SUMO1 targets, overexpression of SUMO1 and Ubc9 could potentially influence many events downstream of p53-dependent transcription (including RNA processing and export as well as translation of the luciferase reporter). This is an important issue because the levels of SUMOlated p53 detected in cells even with overexpression of SUMO1 and/or Ubc9 are only marginal. The most interesting interpretation of the marginal levels of SUMOlated p53 is that this modification is a dynamic process necessary for transient interactions or processes, such as p53 import into or export out of the nucleus. It will be interesting to determine whether SUMO2 or SUMO3 can (also) modify p53. In light of the finding that SUMO2/3 conjugation is induced by stress, DNA damage, and UV (Saitoh & Hinchey 2000), this could be a real possibility.

Mdm 2

Mdm2 is a RING finger E3 ubiquitin ligase involved in p53 degradation. Inspired by the existence of aberrantly migrating forms of Mdm2 in Western blot analysis and by the fact that an other RING finger protein, PML, is a target for SUMOlation, Buschmann and coworkers hypothesized that Mdm2 may also be modified by SUMO1. Using both immunoprecipitation experiments from human cell extracts and in vitro modification assays, they found that this is indeed the case (Buschmann et al 2000). Interestingly, SUMOlation of Mdm2 seems to have a positive effect on its ability to ubiquitinate p53.

A single point mutation in the RING finger domain of human Mdm2, Lys 446 Arg, prevents not only SUMOlation but also ubiquitination of Mdm2. Although this implies that SUMOlation can protect Mdm2 from ubiqutination, it remains to be verified that the mutation does not interfere with the RING finger structure of Mdm2 or with interactions with the respective E2 enzymes. Somewhat surprisingly, the amino acids surrounding Lys 446 do not match the consensus for SUMO modification (see below), and an other lysine residue that would fit into this consensus (Lys 346) was not investigated.

Mdm2 seems to be predominantly in the SUMOlated form under normal growth conditions, but treatment of cells with either UV light or γ irradiation leads to a rapid loss of the modification. Because the same treatment has long been known to cause massive accumulation of p53, the authors propose the following model:

Under normal growth conditions SUMO1 keeps Mdm2 in a stable and active mode. Consequently, p53 is efficiently degraded. DNA damage-induced demodification of Mdm2 leads to its ubiquitination and subsequent degradation, and, as a consequence, p53 is no longer degraded.

c-Jun

The transcription factor c-Jun is one of the many proteins that interact with Ubc9 in two-hybrid interaction screens (Göttlicher et al 1996). Müller and coworkers (Müller et al 2000) investigated a putative modification of c-Jun with SUMO1. As it turned out, c-Jun is indeed modified with SUMO1 both in vivo and in vitro. In contrast to the modification of IκBα, c-Jun SUMOlation does not interfere with its ubiquitination; moreover, in overexpression experiments both ubiquitination and SUMOlation seem to be negatively regulated by stress-induced phosphorylation. How ubiquitination and SUMOlation of c-Jun are than separately controlled in vivo remains one of the key questions.

Homeodomain-Interacting Protein Kinase 2

Homeodomain-interacting protein kinases (HIPKs) are nuclear protein kinases that differentially interact with homeodomain transcription factors (Kim et al 1999). HIPK2 can act as a transcriptional corepressor for homeoproteins. Transfection of a GFP-HIPK2 fusion protein indicates that this protein localizes in nuclear speckles that are distinct from PD10 bodies (PODs). A nuclear speckle retention signal was identified in HIPK2 and shown to overlap with a domain rich in prolines, glutamates, aspartates, serines, and threonines (a so-called PEST element; see below). This sequence was subsequently used in yeast two-hybrid interaction studies, which led to the identification of Ubc9 as a binding partner for HIPK2. Overexpression of both SUMO1 and HIPK2 results in SUMOlation of HIPK2, presumably at lysine 1182, and both proteins colocalize to nuclear speckles. Deletion of the last 30 amino acids (including lysine 1182) or mutation of lysine 1182 to an arginine results in a diffuse nuclear distribution of HIPK2 by immunofluorescence and loss of the SUMOlated species in Western blot analysis. These results indicate that SUMOlation plays a role in targeting HIPK2 to subnuclear compartments (Kim et al 1999).

Drosophila Tramtrack 69

The *Drosophila tramtrack* (*ttk*) gene encodes two proteins by alternative splicing, Ttk69 and Ttk88. These proteins are transcriptional repressors of neuronal cell differentiation and play a role in photoreceptor cell development in the *Drosophila* compound eye. Ttk88 interacts with the RING finger protein Seven in absentia (Sina), which promotes its degradation by the ubiquitin pathway (Li et al 1997). Sina, on the other hand, interacts with dUbc9 in two-hybrid interaction screens (Hu et al 1997). Since RING finger proteins have recently been implicated as

ubiquitin-ligating (E3) enzymes (Lorick et al 1999), Lehembre and co-workers (Lehembre et al 2000) speculated that Sina may function as a SUMO-ligating enzyme in SUMOlation of Ttk88 and Ttk69. To test this hypothesis, they determined whether Ttks can be modified by *Drosophila* SUMO2 in transfection experiments. This turned out to be the case for Ttk69 but not for Ttk88. Western blot analysis of untransfected cells also indicates the presence of a SUMO2-modified form of Ttk69, and immunofluorescence analysis on third-instar polytene chromosomes from salivary glands reveals a partial overlap of Ttk69 and SUMO2 localization. Both modified and unmodified Ttk69 bind to DNA containing a Ttk69-binding site but not to unrelated DNA in an in vitro binding assay. These findings indicate that SUMOlation of Ttk69 is not required for DNA binding per se; understanding its functional significance will have to await further analysis. Whether the original hypothesis, the implication of Sina in the SUMOlation of Ttks, is correct remains to be seen. The apparent absence of Ttk88 SUMOlation speaks against this idea because the Sina/Ttk interaction domain is identical in both proteins.

Topoisomerase I

DNA topoisomerase I (TOP1) is needed for relaxation of supercoiled DNA and is implicated in the recognition of DNA lesions. A crucial reaction intermediate during relaxation of DNA is the formation of a DNA-topoisomerase I complex (the cleavable complex) where topoisomerase I is covalently linked to a 3′ end of DNA, thereby creating a single-stranded DNA break. Cleavable complexes are also formed in the vicinity of DNA lesions and in the presence of the antitumor agent, camptothecin (CPT). Although formation of cleavable complexes may be necessary for the initial stages of the DNA damage response, these complexes are also potentially dangerous to the cell because they can mediate illegitimate recombination, which can lead to genomic instability and oncogenesis (Larsen & Gobert 1999). It has been known for some time that CPT induces rapid covalent modification of human topoisomerase I. This modification has been presumed to be ubiquitination because TOP1-mediated DNA damage can induce ubiquitin/proteasome-dependent degradation of TOP1. As it turns out, CPT-dependent modification of TOP1 is the result of SUMOlation rather than ubiquitination (Mao et al 2000). Addition of CPT to mammalian cells leads to the rapid appearance (within 15 min) of a protein ladder that reacts with both anti-TOP1 and anti-SUMO1 antibodies, indicating that TOP1 is modified with several SUMO1 molecules. Stable overexpression of Ubc9 in these cells increases the levels of these modified species. That this modification in fact results from SUMOlation was further confirmed by the demonstration that human TOP1 is also a target for SUMO (Smt3) modification in yeast. Again the modification was induced by the addition of CPT and depended on both functional Smt3 and Ubc9.

Two aspects of these findings are particularly remarkable. First, this study presents the first demonstration of a very rapid, DNA damage-dependent, induction of SUMO1-modification (see above). Second, the finding that human

topoisomerase I is also a substrate for *S. cerevisiae* SUMO modification emphasizes the functional homology between human SUMO1 and its *S. cerevisiae* homolog Smt3.

Glut1 and Glut4

Whereas most SUMO1 targets seem to be involved in nuclear functions, two plasma membrane-spanning proteins have also recently been linked to the SUMO pathway. These are the glucose transporters Glut1 and Glut4 (Giorgino et al 2000). In insulin-sensitive tissues, a significant fraction of Glut1 resides at the plasma membrane even in the absence of insulin and is mainly responsible for basal glucose transport. In contrast, Glut4 is responsible for the insulin-dependent increase in glucose transport. Insulin causes recruitment of Glut4 from intracellular tubovesicular structures to the plasma membranes. Giorgino and coworkers identified Ubc9 as a binding partner for Glut4 in the yeast two-hybrid system and confirmed this interaction subsequently by pull-down assays. Stable overexpression of wt Ubc9 causes significant changes in the levels of Glut1 and Glut4 and alters both basal and insulin-stimulated glucose transport. Whether the effects caused by Ubc9 overexpression are through SUMOlation of the glucose transporters remains to be seen. Although it has been reported that these receptors are modified by SUMO, the evidence for this is still rather preliminary (based solely on IP-Western blot experiments).

Yeast Septins Cdc3, Cdc11, and Sep7

The only known targets for SUMO conjugation in yeast are the septins Cdc3 (Johnson & Blobel 1999, Takahashi et al 1999), Cdc11, and Shs1/Sep7 (Johnson & Blobel 1999). These proteins belong to a family of GTP-binding coiled-coil proteins that are involved in bud neck formation and bud site selection, but their molecular functions are not clear. Of the septin genes, only *CDC3* is absolutely essential for growth, *CDC10* and *CDC11* are required for viability in many but not all strain backgrounds, and *SEP7* is not essential (reviewed in Field & Kellogg 1999). In an extensive study, Johnson & Blobel showed that Cdc3, Cdc11, and Sep7 are the major targets for SUMOlation in mitosis and subsequently mapped seven different SUMOlation sites in these three proteins. Surprisingly, a yeast strain lacking all seven SUMOlation sites grew at wt rates, mated, and sporulated with wt efficiency, and showed no hypersensitivity to a number of different stress conditions. In wt cells, SUMOlation of the septins appears to occur shortly before the onset of anaphase, at which time an intensive staining of SUMO can be observed at the bud neck. This staining is dramatically reduced in the mutant, but the septins are still present at the bud neck. The only discernible phenotype was a deficiency in disassembly of the septin rings after cytokinesis, which led to the appearance of extra septin rings in virtually all budded cells. These findings suggest that SUMOlation plays a (nonessential) role in septin dynamics.

Potential SUMO Targets

Several of the verified SUMO targets (e.g. PML, IκBα, p53, and HIPK2) were initially linked to the SUMOlation pathway through identification of Ubc9 and/or SUMO1 as binding partners in yeast two-hybrid interaction screens. In hindsight, this interaction may be explained by the fact that yeast contains enzymes necessary for covalent coupling of proteins with SUMO1. Interestingly, a large number of proteins listed in Table 2 interact with Ubc9 or SUMO1 in the two-hybrid system. Given the interaction of bona fide SUMO targets in this screen, one should consider these proteins as potential targets for SUMOlation. Some of these proteins also interact with Ubc9 in vitro, but it is not always clear whether these interactions are direct or mediated by additional factors present in the extracts. However, Ubc9 seems to bind directly to at least one bona fide SUMO target because a heterotrimeric complex consisting of RanGAP, RanBP2, and Ubc9 can be precipitated from solubilized nuclear envelopes (Lee et al 1998). Aside from the possibility that Ubc9/SUMO1-interacting proteins are SUMO targets, other possibilities should be considered. For example, SUMO1-interacting proteins may not be modified themselves but could well be binding partners for SUMOlated proteins. Moreover, Ubc9-interacting proteins could potentially be components of SUMO-ligating enzymes.

MOTIFS FOR SUMOLATION

Many SUMO Targets Contain PEST Sequences

No obvious sequence homology domains can be identified among verified and potential SUMO targets. However, Kim and co-workers recently found that the minimal Ubc9-interacting domain (amino acids 860–894) of the SUMO target HIPK2 maps to its PEST element and pointed out that many of the putative and bona fide SUMO targets contain similar sequences (Kim et al 1999). PEST sequences are defined as stretches of at least 12 amino acids rich in proline, glutamate, aspartate, serine, and threonine residues and lacking positively charged amino acids (Rechsteiner & Rogers 1996).

We analyzed all proteins listed in Tables 1 and 2 with the PEST-Find algorithm developed by Rechsteiner & Rogers (www.at.embnet.org/embnet/tools/bio/PESTfind/). Indeed, of the 19 verified SUMO targets, 10 contain one or more strong PEST sequence. Of the 27 putative targets, 7 also have a strong PEST sequence. Considering that only about 10% of all proteins contain PEST sequences (Rechsteiner & Rogers 1996), this may be more than just a coincidence. For a number of reasons, it seems unlikely that PEST elements represent recognition signals for SUMOlating enzymes. Even though the PEST sequence of HIPK2 is required for the interaction with Ubc9 in yeast, this is not true for the two-hybrid interactions of PML, p53, and IκBα with Ubc9 (Desterro et al 1998, Duprez et al 1999, Gostissa et al 1999). In all three cases, the minimal domain required for the two-hybrid interaction lies outside the PEST sequences. In fact, deletion of the

TABLE 2 Ubc9 and/or SUMO interacting proteins—potential targets for SUMOlation

Protein	Two hybrid with	PEST sequence[a]	References
Rad51	Ubc9 SUMO1	–	Kovalenko et al 1996, Shen et al 1996a Shen et al 1996b
Rad52	Ubc9 SUMO1	0	Shen et al 1996a,b
Fas/Apo receptor	Ubc9 SUMO1	–	Becker et al 1997, Okura et al 1996 Wright et al 1996
TNF-α receptor	Ubc9 SUMO1	–	Okura et al 1996, Saltzman et al 1998
Ethylene-inducing xylanase	SUMO	–	Hanania et al 1999
Glucocorticoid receptor	Ubc9	+	Göttlicher et al 1996
Papillomavirus E1 protein	Ubc9	–	Yasugi & Howley 1996
Wilms' tumor gene product	Ubc9	–	Wang et al 1996
Adenovirus E1A	Ubc9	+	Hateboer et al 1996
E2A proteins	Ubc9	+	Kho et al 1997, Loveys et al 1997
TEL (ETV6)	Ubc9	0	Chakrabarti et al 1999
ATF2	Ubc9	+	Firestein & Feuerstein 1998
ETS-1	Ubc9	–	Hahn et al 1997
D. melanogaster Hsp23, Hsp27	Ubc9	–	Joanisse et al 1998
Bleomycin hydrolase	Ubc9	–	Koldamova et al 1998
Poly(ADP-ribose) polymerase	Ubc9	0	Masson et al 1997
Androgen receptor	Ubc9	+	Poukka et al 1999
MEKK1	Ubc9	–	Saltzman et al 1998
Cor1	Ubc9	–	Tarsounas et al 1997
Syn1	Ubc9	–	Tarsounas et al 1997
S. pombe PCNA	SUMO	–	Tanaka et al 1999
S. cerevisiae Cbf3p	UBC9	+	Jiang & Koltin 1996
D. melanogaster Sina	UBC9	0	Hu et al 1997
Human Siah-1, hSiah-2	–		

[a]As determined by the PEST-Find algorithm developed by Rechsteiner & Rogers (www.at.embnet.org/embnet/tools/bio/PEST-find/).
+ indicates at least one PEST-like sequence with a score above +5.
0 indicates at least one PEST-like sequence with a score between 0 and 5.
– indicates a score below 0.

PEST sequence in PML even enhances the two-hybrid interaction with SUMO1 (Duprez et al 1999). Moreover, the smallest domains of RanGAP1 (amino acids 502–592; Matunis et al 1998), p53 (amino acids 294–393; Gostissa et al 1999), and IκBα (amino acids 1–256; Desterro et al 1998) that are sufficient for SUMOlation do not have PEST sequences.

However, PEST sequences are rich in SP/TP motifs that are minimal recognition elements for serine/threonine kinases. Therefore, it is possible that the PEST elements found in SUMO targets represent regulatory elements that, depending on their phosphorylation state, allow or prevent the modification. Precedence for such an interpretation comes from the ubiquitin field. For example, multiple phosphorylations within PEST elements are required for ubiquitin-dependent degradation of the yeast G1 cyclins Cln3 and Cln2 (reviewed in Hershko & Ciechanover 1998). An alternative explanation for the large number of SUMO targets with PEST elements is that most SUMO targets are also substrates for PEST element–dependent degradation by the ubiquitin/proteasome pathway. Ubiquitin/proteasome-dependent degradation has been shown for several of the verified SUMO1 targets (e.g. PML, IκBα, and p53). It will be interesting to see whether other PEST element-containing SUMO1 targets are subject to phosphorylation and/or ubiquitin-dependent degradation.

A Minimal Consensus Site for Modification by SUMO

For a number of SUMO targets the lysine acceptor sites have been mapped by mutagenesis of putative acceptor lysines to arginines and subsequent analysis of the modification state of these mutants in vivo. However, whereas the lysine-to-arginine mutation is unlikely to cause structural problems, other caveats of this method should be taken into consideration. Absence of SUMOlation could be caused either by a lack of the acceptor site or by the inability of the mutant protein to interact with its SUMOlating enzymes. Only two acceptor sites (one in RanGAP1 and one in Cdc3) have been verified by peptide sequencing and mass spectroscopy. With this in mind one should treat the sequence comparison of published acceptor sites (Table 3) with caution. Alignment of the sequences surrounding SUMO acceptor sites reveals a minimal consensus sequence, aKX(E,D). In addition, most sequences contain proline and/or glycine residues 2 to 5 amino acids upstream or downstream of the acceptor lysine. These may help to insure accessibility of the substrate for the conjugation apparatus by introducing a turn close to the acceptor lysine. Two out of the three sequences lacking glycine or proline residues lie at the very N- or C-terminal ends of the protein and could therefore be easily accessible.

CONCLUSIONS

SUMO conjugation and deconjugation are highly dynamic processes. The equilibrium between the modified and unmodified form of any given target can apparently be determined by several key features: the availability of free SUMO, the activity

TABLE 3 Comparison of SUMO acceptor sites

Acceptor site		Flanking sequences	References
RanGAP1	K526[a]	HMGLL**K**SEDKV	Mahajan et al 1998, Matunis et al 1998
PML	K65	CQAEA**K**CPKLL	Duprez et al 1999, Kamitani et al 1998a
	K160	HQWFL**K**HEARP	
	K490	PRKVI**K**MESEE	
IκBα	K21	PRDGL**K**KERLL	Desterro et al 1998
p53	K386	KKLMF**K**TEGPD	Gostissa et al 1999, Rodriguez et al 1999, Müller et al 2000
Sp100	K297	RLVDI**K**KEKPF	Sternsdorf et al 1999
HIPK2	K1182	PLSPA**K**VNQYP	Kim et al 1999
Cdc3	K4	––MSL**K**EEQVS	Johnson & Blobel 1999
	K11[a]	EQVSI**K**QDPEQ	
	K30	NDVQI**K**QESQD	
	K63	AESDV**K**VEPGL	
Cdc11	K412	KEAKI**K**QEE––	Johnson & Blobel 1999
Sep7/Shs1	K426	LGREI**K**QENEN	Johnson & Blobel 1999
	K437	LIRSI**K**TESSP	
CMV IE2	K175	MLPLI**K**QEDIK	Hofmann et al 2000
	K180	KQEDI**K**PEPDF	
c-Jun	K229	RLQAL**K**EEPQT	Müller et al 2000
Mdm2	K446	CQGRP**K**NGCIV	Buschmann et al 2000
Consensus		a**K**xE[b]	

[a]Identified by mass spectrometry.

[b]K indicates the lysine residue that serves as the SUMO attachment site;
a stands for an aliphatic amino acid residue; x stands for any amino acid residue.

and localization of conjugating and deconjugating enzymes, the presence of binding partners that may protect the conjugate from isopeptidases, and alterations in the target proteins (for example, by phosphorylation and dephosphorylation) that regulate their availability for modifying and demodifying enzymes. Two modes of function for SUMOlation have been suggested: a role in the regulation of protein/protein interactions (often leading to altered subcellular localizations) and a role as an antagonist of ubiquitin (Figure 4). Both functions are plausible and they are obviously not mutually exclusive.

Looking at the list of verified and potential SUMO targets, one can note an obvious accumulation of proteins involved in DNA replication and repair [Rad51, Rad52, PCNA, Poly (ADP-ribose) polymerase, and topoisomerase], mitosis

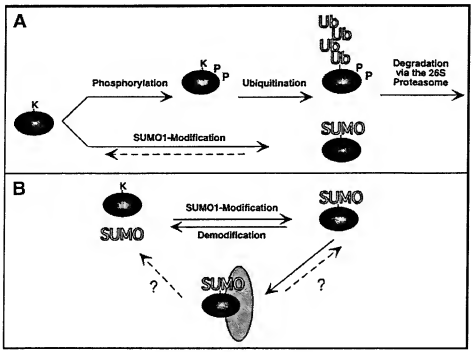

Figure 4 Two predicted modes for SUMO function. (*A*) SUMO as an antagonist to ubiquitin. Where SUMOlation and ubiquitination are mutually exclusive, SUMO may protect its target from ubiquitin-dependent degradation. (*B*) SUMO as a regulator of protein/protein interactions. SUMOlation increases the affinity of the target protein to its binding partner.

(Cbf3p, Cdc3, Cdc11, Sep7), and particularly in signal transduction (e.g. p53, HIPK2, IκBα, tramtrack 69, glucocorticoid and androgen receptors, c-Jun, E2A, TEL, Sina, and Fas/Apo- and TNFα receptor). Even RanGAP1 and RanBP2 could be considered part of the signal transduction group because they are involved in the translocation of signaling factors into and out of the nucleus. Whether the connection of SUMO to just a few specific biological pathways has functional implications or simply reflects the predominant areas of current reseach remains to be seen. In either case, given that only a small fraction of the physiological SUMO targets have been identified, the SUMO field promises to develop into a "heavy weight" in the years to come.

ACKNOWLEDGMENTS

I thank Drs. Jürgen Dohmen, Mark Hochstrasser, Hisatoh Saitoh, and Chin Ha Chung for generously sharing results prior to publication, and Tanja Büsgen as well as Dr. Sowmya Swaminathan for critical reading of the manuscript. Many thanks also go to Dr. Ludger Hengst and the members of my group for many stimulating discussions. Research in my laboratory is supported by the Bundesministerium für Bildung und Forschung (BMBF) and the Peter and Traudl Engelhorn Stiftung.

LITERATURE CITED

al-Khodairy F, Enoch T, Hagan IM, Carr AM. 1995. The *Schizosaccharomyces pombe hus5* gene encodes a ubiquitin conjugating enzyme required for normal mitosis. *J. Cell Sci.* 108:475–86

Bayer P, Arndt A, Metzger S, Mahajan R, Melchior F, et al. 1998. Structure determination of the small ubiquitin-related modifier SUMO-1. *J. Mol. Biol.* 280:275–86

Becker K, Schneider P, Hofmann K, Mattmann C, Tschopp J. 1997. Interaction of Fas(Apo-1/CD95) with proteins implicated in the ubiquitination pathway. *FEBS Lett.* 412:102–6

Bhaskar V, Valentine SA, Courey AJ. 2000. A functional interaction between dorsal and components of the Smt3 conjugation machinery. *J. Biol. Chem.* 275:4033–40

Boddy MN, Howe K, Etkin LD, Solomon E, Freemont PS. 1996. PIC 1, a novel ubiquitin-like protein which interacts with the PML component of a multiprotein complex that is disrupted in acute promyelocytic leukaemia. *Oncogene* 13:971–82

Bonifacino JS, Weissman AM. 1998. Ubiquitin and the control of protein fate in the secretory and endocytic pathways. *Annu. Rev. Cell Dev. Biol.* 14:19–57

Buschmann T, Fuchs SY, Lee CC, Pan ZQ, Ronai Z. 2000. SUMO-1 modification of Mdm2 prevents its self-ubiquitination and increases Mdm2 ability to ubiquitinate p53. *Cell.* 101:753–62

Chakrabarti SR, Sood R, Ganguly S, Bohlander S, Shen Z, Nucifora G. 1999. Modulation of TEL transcription activity by interaction with the ubiquitin-conjugating enzyme UBC9. *Proc. Natl. Acad. Sci. USA* 96:7467–72

Chelbi-Alix MK, de The H. 1999. Herpes virus induced proteasome-dependent degradation of the nuclear bodies-associated PML and Sp100 proteins. *Oncogene* 18:935–41

Chen A, Mannen H, Li SS. 1998. Characterization of mouse ubiquitin-like SMT3A and SMT3B cDNAs and gene/pseudogenes. *Biochem. Mol. Biol. Int.* 46:1161–74

Choudhury BK, Li SS. 1997. Identification and characterization of the SMT3 cDNA and gene from nematode *Caenorhabditis elegans*. *Biochem. Biophys. Res. Commun.* 234:788–91

Chung CH, Baek SH. 1999. Deubiquitinating enzymes: their diversity and emerging roles. *Biochem. Biophys. Res. Commun.* 266:633–40

Ciechanover A. 1998. The ubiquitin-proteasome pathway: on protein death and cell life. *EMBO J.* 17:7151–60

Collins C, Rommens JM, Kowbel D, Godfrey T, Tanner M, et al. 1998. Positional cloning of ZNF217 and NABC1: genes amplified at 20q13.2 and overexpressed in breast carcinoma. *Proc. Natl. Acad. Sci. USA* 95:8703–8

del Olmo M, Mizrahi N, Gross S, Moore CL. 1997. The Uba2 and Ufd1 proteins of *Saccharomyces cerevisiae* interact with poly(A) polymerase and affect the polyadenylation activity of cell extracts. *Mol. Gen. Genet.* 255:209–18

Desterro JM, Rodriguez MS, Hay RT. 1998. SUMO-1 modification of IκBα inhibits NF-κB activation. *Mol. Cell* 2:233–39

Desterro JM, Rodriguez MS, Kemp GD, Hay RT. 1999. Identification of the enzyme required for activation of the small ubiquitin-like protein SUMO-1. *J. Biol. Chem.* 274:10618–24

Dohmen RJ, Stappen R, McGrath JP, Forrova H, Kolarov J, et al. 1995. An essential yeast gene encoding a homolog of ubiquitin-activating enzyme. *J. Biol. Chem.* 270:18099–109

Duprez E, Saurin AJ, Desterro JM, Lallemand-Breitenbach V, Howe K, et al. 1999. SUMO-1 modification of the acute promyelocytic leukaemia protein PML: implications for nuclear localisation. *J. Cell Sci.* 112:381–93

Epps JL, Tanda S. 1998. The *Drosophila semushi* mutation blocks nuclear import of bicoid during embryogenesis. *Curr. Biol.* 8:1277–80

Everett RD, Freemont P, Saitoh H, Dasso M, Orr A, et al. 1998. The disruption of ND10 during herpes simplex virus infection correlates with the Vmw110- and proteasome-dependent loss of several PML isoforms. *J. Virol.* 72:6581–91

Everett RD, Lomonte P, Sternsdorf T, van Driel R, Orr A. 1999. Cell cycle regulation of PML modification and ND10 composition. *J. Cell Sci.* 112:4581–88

Field CM, Kellogg D. 1999. Septins: cytoskeletal polymers or signalling GTPases? *Trends Cell Biol.* 9:387–94

Firestein R, Feuerstein N. 1998. Association of activating transcription factor 2 (ATF2) with the ubiquitin-conjugating enzyme hUBC9. Implication of the ubiquitin/proteasome pathway in regulation of ATF2 in T cells. *J. Biol. Chem.* 273:5892–902

Giaccia AJ, Kastan MB. 1998. The complexity of p53 modulation: emerging patterns from divergent signals. *Genes Dev.* 12:2973–83

Giorgino F, de Robertis O, Laviola L, Montrone C, Perrini S, et al. 2000. The sentrin-conjugating enzyme mUbc9 interacts with GLUT4 and GLUT1 glucose transporters and regulates transporter levels in skeletal muscle cells. *Proc. Natl. Acad. Sci. USA* 97:1125–30

Giraud MF, Desterro JM, Naismith JH. 1998. Structure of ubiquitin-conjugating enzyme 9 displays significant differences with other ubiquitin-conjugating enzymes which may reflect its specificity for SUMO rather than ubiquitin. *Acta Crystallogr. D Biol. Crystallogr.* 54:891–98

Gong L, Li B, Millas S, Yeh ET. 1999. Molecular cloning and characterization of human AOS1 and UBA2, components of the sentrin-activating enzyme complex. *FEBS Lett.* 448:185–89

Gong L, Millas S, Maul GG, Yeh ET. 2000. Differential regulation of sentrinized proteins by a novel sentrin-specific protease. *J. Biol. Chem.* 275:3355–59

Gostissa M, Hengstermann A, Fogal V, Sandy P, Schwarz SE, et al. 1999. Activation of p53 by conjugation to the ubiquitin-like protein SUMO-1. *EMBO J.* 18:6462–71

Göttlicher M, Heck S, Doucas V, Wade E, Kullmann M, et al. 1996. Interaction of the Ubc9 human homologue with c-Jun and with the glucocorticoid receptor. *Steroids* 61:257–62

Haas AL, Siepmann TJ. 1997. Pathways of ubiquitin conjugation. *FASEB J.* 11:1257–68

Hahn SL, Wasylyk B, Criqui-Filipe P, Criqui P. 1997. Modulation of ETS-1 transcriptional activity by huUBC9, a ubiquitin-conjugating enzyme. *Oncogene* 15:1489–95

Hanania U, Furman-Matarasso N, Ron M, Avni A. 1999. Isolation of a novel SUMO protein from tomato that suppresses EIX-induced cell death. *Plant J.* 19:533–41

Hateboer G, Hijmans EM, Nooij JB, Schlenker S, Jentsch S, Bernards R. 1996. mUBC9, a novel adenovirus E1A-interacting protein that complements a yeast cell cycle defect. *J. Biol. Chem.* 271:25906–11

Hershko A, Ciechanover A. 1998. The ubiquitin system. *Annu. Rev. Biochem.* 67:425–79

Hicke L. 1997. Ubiquitin-dependent internalization and down-regulation of plasma membrane proteins. *FASEB J.* 11:1215–26

Hochstrasser M. 1998. There's the rub: a novel ubiquitin-like modification linked to cell cycle regulation. *Genes Dev.* 12:901–7

Hodges M, Tissot C, Freemont PS. 1998. Protein regulation: tag wrestling with relatives of ubiquitin. *Curr. Biol.* 8:R749–52

Hofmann H, Flöss S, Stamminger T. 2000. Covalent modification of the transactivator protein IE-p86 of human cytomegalovirus by conjugation to the ubiquitin-homologous protein SUMO1 and hSMT3b. *J. Virol.* 74: 2510–24

Howe K, Williamson J, Boddy N, Sheer D, Freemont P, Solomon E. 1998. The ubiquitin-homology gene PIC1: characterization of

mouse (Pic1) and human (UBL1) genes and pseudogenes. *Genomics* 47:92–100

Hu G, Zhang S, Vidal M, Baer JL, Xu T, Fearon ER. 1997. Mammalian homologs of *seven in absentia* regulate DCC via the ubiquitin-proteasome pathway. *Genes Dev.* 11:2701–14

Huang HW, Tsoi SC, Sun YH, Li SS. 1998. Identification and characterization of the SMT3 cDNA and gene encoding ubiquitin-like protein from *Drosophila melanogaster*. *Biochem. Mol. Biol. Int.* 46:775–85

Ishov AM, Sotnikov AG, Negorev D, Vladimirova OV, Neff N, et al. 1999. PML is critical for ND10 formation and recruits the PML-interacting protein Daxx to this nuclear structure when modified by SUMO-1. *J. Cell Biol.* 147:221–34

Jentsch S, Pyrowolakis G. 2000. Ubiquitin and its kin: How close are the family ties? *Trends Cell Biol.* 10:335–42

Jiang W, Koltin Y. 1996. Two-hybrid interaction of a human UBC9 homolog with centromere proteins of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 251:153–60

Joanisse DR, Inaguma Y, Tanguay RM. 1998. Cloning and developmental expression of a nuclear ubiquitin-conjugating enzyme (DmUbc9) that interacts with small heat shock proteins in *Drosophila melanogaster*. *Biochem. Biophys. Res. Commun.* 244:102–9

Johnson ES, Blobel G. 1997. Ubc9p is the conjugating enzyme for the ubiquitin-like protein Smt3p. *J. Biol. Chem.* 272:26799–802

Johnson ES, Blobel G. 1999. Cell cycle-regulated attachment of the ubiquitin-related protein SUMO to the yeast septins. *J. Cell Biol.* 147:981–94

Johnson ES, Schwienhorst I, Dohmen RJ, Blobel G. 1997. The ubiquitin-like protein Smt3p is activated for conjugation to other proteins by an Aos1p/Uba2p heterodimer. *EMBO J.* 16:5509–19

Johnson PR, Hochstrasser M. 1997. SUMO-1: Ubiquitin gains weight. *Trends Cell Biol.* 7:408–13

Kamitani T, Kito K, Nguyen HP, Wada H, Fukuda-Kamitani T, Yeh ET. 1998a. Identification of three major sentrinization sites in PML. *J. Biol. Chem.* 273:26675–82

Kamitani T, Nguyen HP, Kito K, Fukuda-Kamitani T, Yeh ET. 1998b. Covalent modification of PML by the sentrin family of ubiquitin-like proteins. *J. Biol. Chem.* 273:3117–20

Kho CJ, Huggins GS, Endege WO, Hsieh CM, Lee ME, Haber E. 1997. Degradation of E2A proteins through a ubiquitin-conjugating enzyme, UbcE2A. *J. Biol. Chem.* 272:3845–51

Kim KI, Baek SH, Nishimori S, Suzuki T, Uchida S, et al. 2000. A new SUMO-1-specific protease, SUSP1, that is highly expressed in reproductive organs. *J. Biol. Chem.* 275:14102–6

Kim YH, Choi CY, Kim Y. 1999. Covalent modification of the homeodomain-interacting protein kinase 2 (HIPK2) by the ubiquitin-like protein SUMO-1. *Proc. Natl. Acad. Sci. USA* 96:12350–55

Koldamova RP, Lefterov IM, DiSabella MT, Lazo JS. 1998. An evolutionarily conserved cysteine protease, human bleomycin hydrolase, binds to the human homologue of ubiquitin-conjugating enzyme 9. *Mol. Pharmacol.* 54:954–61

Kovalenko OV, Plug AW, Haaf T, Gonda DK, Ashley T, et al. 1996. Mammalian ubiquitin-conjugating enzyme Ubc9 interacts with Rad51 recombination protein and localizes in synaptonemal complexes. *Proc. Natl. Acad. Sci. USA* 93:2958–63

Kretz-Remy C, Tanguay RM. 1999. SUMO/sentrin: protein modifiers regulating important cellular functions. *Biochem. Cell Biol.* 77:299–309

Lammer D, Mathias N, Laplaza JM, Jiang W, Liu Y, et al. 1998. Modification of yeast Cdc53p by the ubiquitin-related protein rub1p affects function of the SCFCdc4 complex. *Genes Dev.* 12:914–26

Lapenta V, Chiurazzi P, van der Spek P, Pizzuti A, Hanaoka F, Brahe C. 1997. SMT3A, a human homologue of the *S. cerevisiae* SMT3 gene, maps to chromosome 21qter and

defines a novel gene family. *Genomics* 40:362–66

Larsen AK, Gobert C. 1999. DNA topoisomerase I in oncology: Dr. Jekyll or Mr. Hyde? *Pathol. Oncol. Res.* 5:171–78

Lee GW, Melchior F, Matunis MJ, Mahajan R, Tian Q, Anderson P. 1998. Modification of Ran GTPase-activating protein by the small ubiquitin-related modifier SUMO-1 requires Ubc9, an E2-type ubiquitin-conjugating enzyme homologue. *J. Biol. Chem.* 273:6503–7

Lehembre F, Badenhorst P, Muller S, Travers A, Schweisguth F, Dejean A. 2000. Covalent modification of the transcriptional repressor Tramtrack by the ubiquitin-related protein smt3 in Drosophila flies. *Mol. Cell Biol.* 20:1072–82

Levine AJ. 1997. p53, the cellular gatekeeper for growth and division. *Cell* 88:323–31

Li H, Leo C, Zhu J, Wu X, O'Neil J, et al. 2000. Sequestration and inhibition of Daxx-mediated transcriptional repression by PML. *Mol. Cell Biol.* 20:1784–96

Li S, Li Y, Carthew RW, Lai ZC. 1997. Photoreceptor cell differentiation requires regulated proteolysis of the transcriptional repressor Tramtrack. *Cell* 90:469–78

Li SJ, Hochstrasser M. 1999. A new protease required for cell-cycle progression in yeast. *Nature* 398:246–51

Li SJ, Hochstrasser M. 2000. The yeast ULP2 (SMT4) gene encodes a novel protease specific for the ubiquitin-like Smt3 protein. *Mol. Cell Biol.* 20:2367–77

Liakopoulos D, Doenges G, Matuschewski K, Jentsch S. 1998. A novel protein modification pathway related to the ubiquitin system. *EMBO J.* 17:2208–14

Lin RJ, Egan DA, Evans RM. 1999. Molecular genetics of acute promyelocytic leukemia. *Trends Genet.* 15:179–84

Liu Q, Jin C, Liao X, Shen Z, Chen DJ, Chen Y. 1999. The binding interface between an E2 (UBC9) and a ubiquitin homologue (UBL1). *J. Biol. Chem.* 274:16979–87

Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S, Weissman AM. 1999. RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. *Proc. Natl. Acad. Sci. USA* 96:11364–69

Loveys DA, Streiff MB, Schaefer TS, Kato GJ. 1997. The mUBC9 murine ubiquitin conjugating enzyme interacts with the E2A transcription factors. *Gene* 201:169–77

Mahajan R, Delphin C, Guan T, Gerace L, Melchior F. 1997. A small ubiquitin-related polypeptide involved in targeting RanGAP1 to nuclear pore complex protein RanBP2. *Cell* 88:97–107

Mahajan R, Gerace L, Melchior F. 1998. Molecular characterization of the SUMO-1 modification of RanGAP1 and its role in nuclear envelope association. *J. Cell Biol.* 140:259–70

Mannen H, Tseng HM, Cho CL, Li SS. 1996. Cloning and expression of human homolog HSMT3 to yeast SMT3 suppressor of MIF2 mutations in a centromere protein gene. *Biochem. Biophys. Res. Commun.* 222:178–80

Mao Y, Sun M, Desai, SD, Liu LF. 2000. SUMO-1 conjugation to topoisomerase I: a possible repair response to topoisomerase-mediated DNA damage. *Proc. Natl. Acad. Sci. USA* 2097:4046–51

Masson M, Menissier de Murcia J, Mattei MG, de Murcia G, Niedergang CP. 1997. Poly(ADP-ribose) polymerase interacts with a novel human ubiquitin conjugating enzyme: hUbc9. *Gene* 190:287–96

Matunis MJ, Coutavas E, Blobel G. 1996. A novel ubiquitin-like modification modulates the partitioning of the Ran-GTPase-activating protein RanGAP1 between the cytosol and the nuclear pore complex. *J. Cell Biol.* 135:1457–70

Matunis MJ, Wu J, Blobel G. 1998. SUMO-1 modification and its role in targeting the Ran GTPase-activating protein, RanGAP1, to the nuclear pore complex. *J. Cell Biol.* 140:499–509

Maul GG. 1998. Nuclear domain 10, the site of DNA virus transcription and replication. *BioEssays* 20:660–67

Mayer RJ, Landon M, Layfield R. 1998. Ubiquitin superfolds: intrinsic and attachable regulators of cellular activities? *Fold Des.* 3:R97–99

Meluh PB, Koshland D. 1995. Evidence that the MIF2 gene of *Saccharomyces cerevisiae* encodes a centromere protein with homology to the mammalian centromere protein CENP-C. *Mol. Biol. Cell* 6:793–807

Müller S, Berger M, Lehembre F, Seeler JS, Haupt Y, Dejean A. 2000. C-Jun and p53 activity is modulated by SUMO1 modification. *J. Biol. Chem.* 275: 13321–29

Müller S, Dejean A. 1999. Viral immediate-early proteins abrogate the modification by SUMO-1 of PML and Sp100 proteins, correlating with nuclear body disruption. *J. Virol.* 73:5137–43

Müller S, Matunis MJ, Dejean A. 1998. Conjugation with the ubiquitin-related modifier SUMO-1 regulates the partitioning of PML within the nucleus. *EMBO J.* 17:61–70

Ohsumi Y. 1999. Molecular mechanism of autophagy in yeast, *Saccharomyces cerevisiae*. *Philos. Trans. R Soc. London Ser. B* 354:1577–80; discussion 80–81

Okuma T, Honda R, Ichikawa G, Tsumagari N, Yasuda H. 1999. In vitro SUMO-1 modification requires two enzymatic steps, E1 and E2. *Biochem. Biophys. Res. Commun.* 254:693–98

Okura T, Gong L, Kamitani T, Wada T, Okura I, et al. 1996. Protection against Fas/APO-1-and tumor necrosis factor-mediated cell death by a novel protein, sentrin. *J. Immunol.* 157:4277–81

Pickart CM. 1997. Targeting of substrates to the 26S proteasome. *FASEB J.* 11:1055–66

Poukka H, Aarnisalo P, Karvonen U, Palvimo JJ, Janne OA. 1999. Ubc9 interacts with the androgen receptor and activates receptor-dependent transcription. *J. Biol. Chem.* 274:19441–46

Qi F, Ridpath JF, Berry ES. 1998. Insertion of a bovine SMT3B gene in NS4B and duplication of NS3 in a bovine viral diarrhea virus genome correlate with the cytopathogenicity of the virus. *Virus Res.* 57:1–9

Rechsteiner M, Rogers SW. 1996. PEST sequences and regulation by proteolysis. *Trends Biochem. Sci.* 21:267–71

Rodriguez MS, Desterro JM, Lain S, Midgley CA, Lane DP, Hay RT. 1999. SUMO-1 modification activates the transcriptional response of p53. *EMBO J.* 18:6455–61

Saitoh H, Hinchey J. 2000. Functional heterogeneity of small ubiquitin-related protein modifiers, SUMO-1 versus SUMO-2/3. *J. Biol. Chem.* 275:6252–58

Saitoh H, Pu R, Cavenagh M, Dasso M. 1997a. RanBP2 associates with Ubc9p and a modified form of RanGAP1. *Proc. Natl. Acad. Sci. USA* 94:3736–41

Saitoh H, Pu RT, Dasso M. 1997b. SUMO-1: wrestling with a new ubiquitin-related modifier. *Trends Biochem. Sci.* 22:374–76

Saitoh H, Sparrow DB, Shiomi T, Pu RT, Nishimoto T, et al. 1998. Ubc9p and the conjugation of SUMO-1 to RanGAP1 and RanBP2. *Curr. Biol.* 8:121–24

Saltzman A, Searfoss G, Marcireau C, Stone M, Ressner R, et al. 1998. hUBC9 associates with MEKK1 and type I TNF-alpha receptor and stimulates NFkappaB activity. *FEBS Lett.* 425:431–35

Seufert W, Futcher B, Jentsch S. 1995. Role of a ubiquitin-conjugating enzyme in degradation of S- and M-phase cyclins. *Nature* 373:78–81

Shayeghi M, Doe CL, Tavassoli M, Watts FZ. 1997. Characterisation of *Schizosaccharomyces pombe* rad31, a UBA-related gene required for DNA damage tolerance. *Nucleic Acids Res.* 25:1162–69

Shen Z, Pardington-Purtymun PE, Comeaux JC, Moyzis RK, Chen DJ. 1996a. Associations of UBE2I with RAD52, UBL1, p53, and RAD51 proteins in a yeast two-hybrid system. *Genomics* 37:183–86

Shen Z, Pardington-Purtymun PE, Comeaux JC, Moyzis RK, Chen DJ. 1996b. UBL1, a human ubiquitin-like protein associating with human RAD51/RAD52 proteins. *Genomics* 36:271–79

Sternsdorf T, Jensen K, Reich B, Will H. 1999. The nuclear dot protein sp100, characterization of domains necessary for dimerization, subcellular localization, and modification by small ubiquitin-like modifiers. *J. Biol. Chem.* 274:12555–66

Sternsdorf T, Jensen K, Will H. 1997. Evidence for covalent modification of the nuclear dot-associated proteins PML and Sp100 by PIC1/SUMO-1. *J. Cell Biol.* 139:1621–34

Suzuki T, Ichiyama A, Saitoh H, Kawakami T, Omata M, et al. 1999. A new 30-kDa ubiquitin-related SUMO-1 hydrolase from bovine brain. *J. Biol. Chem.* 274:31131–34

Takahashi Y, Iwase M, Konishi M, Tanaka M, Toh-e A, Kikuchi Y. 1999. Smt3, a SUMO-1 homolog, is conjugated to Cdc3, a component of septin rings at the mother-bud neck in budding yeast. *Biochem. Biophys. Res. Commun.* 259:582–87

Tanaka K, Nishide J, Okazaki K, Kato H, Niwa O, et al. 1999. Characterization of a fission yeast SUMO-1 homologue, Pmt3p, required for multiple nuclear events, including the control of telomere length and chromosome segregation. *Mol. Cell. Biol.* 19:8660–72

Tanaka K, Suzuki T, Chiba T. 1998. The ligation systems for ubiquitin and ubiquitin-like proteins. *Mol. Cell* 8:503–12

Tarsounas M, Pearlman RE, Gasser PJ, Park MS, Moens PB. 1997. Protein-protein interactions in the synaptonemal complex. *Mol. Biol. Cell* 8:1405–14

Tong H, Hateboer G, Perrakis A, Bernards R, Sixma TK. 1997. Crystal structure of murine/human Ubc9 provides insight into the variability of the ubiquitin-conjugating system. *J. Biol. Chem.* 272:21381–87

Tsytsykova AV, Tsitsikov EN, Wright DA, Futcher B, Geha RS. 1998. The mouse genome contains two expressed intronless retroposed pseudogenes for the sentrin/sumo-1/PIC1 conjugating enzyme Ubc9. *Mol. Immunol.* 35:1057–67

Vierstra RD, Callis J. 1999. Polypeptide tags, ubiquitous modifiers for plant protein regulation. *Plant Mol. Biol.* 41:435–42

Vijay-Kumar S, Bugg CE, Cook WJ. 1987. Structure of ubiquitin refined at 1.8 Å resolution. *Mol. Biol.* 194:531–44

Wang ZY, Qiu QQ, Seufert W, Taguchi T, Testa JR, et al. 1996. Molecular cloning of the cDNA and chromosome localization of the gene for human ubiquitin-conjugating enzyme 9. *J. Biol. Chem.* 271:24811–16

Watanabe TK, Fujiwara T, Kawai A, Shimizu F, Takami S, et al. 1996. Cloning, expression, and mapping of UBE2I, a novel gene encoding a human homologue of yeast ubiquitin-conjugating enzymes which are critical for regulating the cell cycle. *Cytogenet. Cell Genet.* 72:86–89

Wilkinson KD. 1997. Regulation of ubiquitin-dependent processes by deubiquitinating enzymes. *FASEB J.* 11:1245–56

Wright DA, Futcher B, Ghosh P, Geha RS. 1996. Association of human fas (CD95) with a ubiquitin-conjugating enzyme (UBC-FAP). *J. Biol. Chem.* 271:31037–43

Yasugi T, Howley PM. 1996. Identification of the structural and functional human homolog of the yeast ubiquitin conjugating enzyme UBC9. *Nucleic Acids Res.* 24:2005–10